

nature

April 15, 1976

Every cow carries a government health warning

CORONARY heart disease (CHD) is a major cause of death in middle and old age in the UK and many other western countries. For instance, in the age range 45–54 years, 39% of all male deaths and 12% of all female deaths are from CHD. Since 1950 death rates from CHD have increased consistently for men; the death rate is 60% higher in that same age range now than it was 25 years ago. Strangely, there is no evidence of a similar trend for women.

In the light of these and similar figures many countries have looked at ways in which the incidence of CHD might be reduced. A working party of the Royal College of Physicians of London and the British Cardiac Society has just issued the latest assessment, which will be distributed widely to general practitioners (*Journal of the Royal College of Physicians*, 10, 213–275). It deserves to be read by scientists too, not just for the factual content but also as an example of a rather good blend of science and commonsense.

The report comes out unambiguously against major investment in more after-the-fact services which “cannot hope to achieve a major reduction in the overall burden of CHD in the community”. Preventive measures against a disease with palpable environmental factors are, however, spelt out in considerable detail. They include stopping smoking, monitoring blood pressure whenever possible, changing diet and increasing physical activity (including “getting breathless some time every day”). Probably the most controversial of these suggestions, all of which have been floating around for a long time, is the fairly explicit talk of dietary adjustments. The recommendations are made, the report says, because plasma lipid levels in the UK indicate a population generally at risk.

Fat in the diet, it is recommended, should be reduced from its present average contribution of 42% of the total calorie intake to around 35%. Saturated fats (which at present constitute half of this contribution) should be particularly reduced, to be replaced in part by polyunsaturated fats. What this means is less meat and egg yolks, more poultry and fish; less butter, more soft margarine high in polyunsaturated fats; corn oil, sunflower oil and safflower oil, not hard margarines and lard; cream and top of the milk are to be avoided, vegetables and fruit encouraged. In fact all that one’s instincts say, except in the case of dairy products.

Readers from overseas, particularly from the United

States and Australia, can be forgiven a certain wry amusement at this point that at last the UK is starting to publicise what has been common knowledge for many years. The word “polyunsaturated”, surely the most scientific word the layman will ever need to take into his vocabulary, was sufficiently well known as early as 1970 for the Massachusetts Heart Association to be distributing leaflets to all homes asking: “Will your children remember your tasty meals, low in saturated fats and cholesterol?”

Why has it not been possible for similar exhortations (perhaps a touch less ham-fisted) to be made in Britain, and why has a major manufacturer of polyunsaturated margarine had till recently to pursue publicity for his product by unconventional means? (Not, it should be said, that it took more than a day after the report’s appearance before perfectly explicit advertisements were appearing.)

Part of the answer may be in genuine scientific doubt, not so much about the role of unsaturated fats in raising plasma cholesterol levels, nor even about the correlation between cholesterol levels and the risk of CHD, but concerning the value of lowering plasma lipid levels by dietary change in trying to reduce CHD. The report itself cautiously concludes that there is “probably sufficient evidence . . . to make dietary recommendations in the hope of affecting a degree of prevention”. Not, it must be admitted, the most gripping of cases.

But it is not the medical science so much as the sacred cow that has held things up. The dairy industry could be hit hard by dietary changes if the nation took them seriously—less meat, less butter, little cream. Small wonder that the issue has been carefully skirted in Whitehall for as long as possible, and small wonder that the National Dairy Council was quickly out fighting.

The publication of this report must not herald the start of some form of public relations war between rival products each of which will doubtless (such is the nature of the scientific literature) be able to marshal cast-iron evidence for its own point of view. The onus is on the Department of Health and Social Security to ensure that knowledge is diffused into the community rather than thrown at the citizen by interested parties. What is needed is a clear statement of the meaning of the scientific words and a relatively low-key assessment of the risks, followed by a requirement that advertisers state the contents of their product simply. □



Taking the naturalness out of natural disasters

Phil O'Keefe, Ken Westgate and Ben Wisner argue the case that disasters are more a consequence of socio-economic than natural factors

THE MEDIA continually present us with graphic accounts of natural disasters—the Bangladesh cyclone, the Nicaraguan earthquake and the African drought are just some of the recent examples of catastrophes that caused much death and destruction. Since the beginning of 1976, we have already had detailed accounts of floods in Venezuela, Australia and Indonesia, famine in Niger, landslides in Ecuador, drought in Malaysia and the mammoth earthquake in Guatemala. It is difficult to gather global information on the frequency and, more importantly, the impact of these disasters. But a set of global statistics from all available resources has recently been compiled.

The Disaster Research Unit at the University of Bradford has collected data from international organisations, government departments, academic institutions and insurance companies. The information was confused because most institutions recording disaster data had an implicit role in the disasters and their aftermath which coloured their recording. For example, the international and governmental organisations were chiefly concerned with disasters in which aid was being donated, academic institutions were primarily interested in recording unusual phenomena which might not necessarily be disastrous, while insurance companies only recorded information directly related to their business. In spite of the data's unreliability, several tendencies can be observed.

The most important tendency is an increase in the occurrence of disasters over the last 50 years. Figure 1 shows this increase from 1947–70 of large-scale disasters, that is, those disasters covering more than a ten-degree square on a world map and where damage exceeds \$1 million. This tendency is paralleled by an increasing loss of life per disaster. The greatest loss of life per disaster is observed in underdeveloped countries, and there are general indications that the vulnerability of these countries in particular is increasing. Such conclusions clearly

require further explanation.

Disaster marks the interface between an extreme physical phenomenon and a vulnerable human population. It is of paramount importance to recognise both of these elements. Without people there is no disaster. The two elements are basic to an explanation of an increase in the occurrence of disasters. No major geological or climatological changes over the last 50 years adequately explain the rise. There is little argument about geological change, but there has been much mystifying argument about climatic change, especially following the prolonged drought over the African and Asian continents. But no firm conclusion can be drawn about changing climatic conditions from available evidence. Randall Baker at the Development Studies School of the University of East Anglia recently reviewed all the evidence of climatic change in Africa, and offered the Scottish judgment of "case not proven". Even if some long-term change was observable it would not explain the increase in disaster occurrence observed in the data.

If it is accepted that there has been no major geological and climatological change in recent years, then it can be assumed that the probability of the extreme physical occurrence is constant. If the probability is constant, then logically the explanation of the increasing numbers of disasters must be sought in an explanation of the growing vulnerability of the population to extreme physical events. Ongoing research suggests that some radical rethinking on the nature of "natural" disasters is necessary.

It is known that the frequency of natural disasters is increasing especially in underdeveloped countries. Indeed, the increased vulnerability of people to extreme physical events can be seen as intimately connected with the continuing process of underdevelopment recorded throughout the world. As population continues to expand, and as resources continue to be controlled by a minority, the real standard of living drops for much of the world's population. This population is increasingly vulnerable to environmental variation as the process continues. Paul Richards of the Environmental Unit in the International African Institute recently

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emphasised this in his introduction to *African Environment: Problems and Perspectives*: "... just as natural processes such as lack of rainfall affect social structures", he argued, "so social processes such as economic 'development' can affect natural systems, 'causing' famine and soil erosion for example". He went on:

"[I]n a continent where international ties of dependency, massive international labour migration and multinational companies prevail and in a world where growth does not necessarily mean development, and development does not necessarily bring enrichment or an increase in personal happiness, the ultimate cause of environmental problems may well be traceable to the structural imbalances between rich and poor countries and we would be right to replace the term *natural* with the more appropriate term *social* or *political* disaster".

These suggestions would strike the Guatemalan peasant as commonsense. The recent earthquake there is no longer identified as a natural event—the local inhabitants who survived are referring to the event as a "classquake". It is a view which reflects their broad experience. That experience is the basis for an explanation of their general plight in terms of a process, not of development, but of underdevelopment—a process which their increasing vulnerability reflects. Instead of moving independently (as one school of thought argues) from a state of underdevelopment to development along the lines that the now-developed west has already done, Third World countries (says an alternative, radically opposed school) have in fact been moving retrogressively from a state of underdevelopment to one of underdevelopment, in a process of "marginalisation" which is not so much separate from as the price of the west's development in an increasingly inter-

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dependent world. As this has happened, the relatively deprived sectors of the populations within these countries have become even worse off.

The reality of this situation is shown in Figure 2. It is the poorest countries which tend to suffer most disaster strikes. This point ought to encourage precautionary planning to mitigate the effect of future disaster. Such precautionary planning needs to be totally integrated into planning for real development, which means the necessary concentration on the vulnerability of a population to future disaster can only be done successfully through an understanding of the marginalisation process. Emphasis on precautionary planning does not, of course, make unnecessary the valuable action invariably mounted in the aftermath of a disaster: in fact, the formation of the London Technical Group, which supplies accurate technical reports on the position after disasters and generally provides expertise on relief measures, is to be warmly welcomed. In the long run, however, precautionary planning would be more beneficial than relief work, since it would aim to consider and alleviate the causes and not merely the symptoms of disaster.

Successful precautionary planning, then in focusing on the population's vulnerability, depends upon the identi-

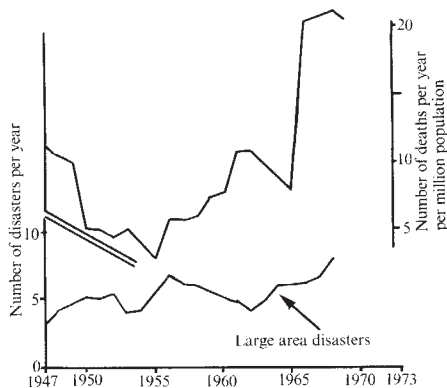


Fig. 1 Global disasters 1947-1973: Five-year moving average.

cation of cultural attitudes towards the use of indigenous resources at local and regional levels, and the incorporation into development planning of strategies to mitigate disasters; precautionary planning should be seen as the insurance chapter in any development plan. The aim would be to raise the standards of life of people currently ill-placed to resist disasters because those standards are too low. The average "cost" of a disaster strike, a rather meaningless concept when the range of impact is so great, is about \$20 million. It is sufficient to say that

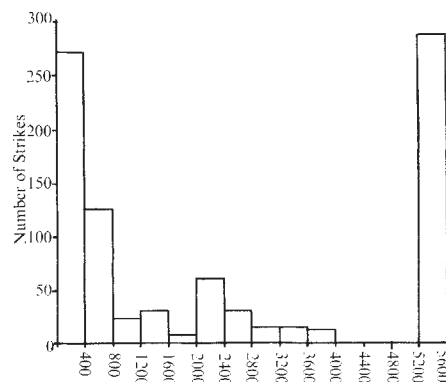


Fig. 2 Number of disaster strikes by per capita income of disaster-strike area: the large number of disasters recorded in developed countries is a reflection of the closer monitoring of disaster events.

more than \$1,100 million was spent on disaster assistance in 1973; and 96 countries of the world had less than this amount of resource capital as GNP in 1973. The time is ripe for some form of precautionary planning which considers vulnerability of the population as the real cause of disaster—a vulnerability that is induced by socio-economic conditions that can be modified by man, and is not just an act of God. Precautionary planning must commence with the removal of concepts of naturalness from natural disasters. □

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USA

High technology protectionism

Colin Norman reports from Washington on US efforts to control the export trade in strategic technology being stimulated by the era of "détente"

A REPORT which has so far received scant public attention, but which is now being closely studied in the Pentagon, contains a number of startling and disturbing recommendations for controlling trade in high technology goods between western nations and the Soviet Union. Prominent among them is the suggestion that the United States should apply tough sanctions against any country—including America's European allies—which passes potentially strategic technology to a communist country. It also recommends that the Department of Defense should keep a close watch on government-to-government scientific exchange agreements and that it should keep its eye on the training of people from communist countries at major institutes and universities in the United States.

The report, published by the Defense Science Board (DSB), a top-level Pentagon advisory group, is the latest in a series of outpourings of concern that the United States' lead over the Soviet Union in strategic areas of technology is gradually being whittled away by commercial sales of computers, electronic goods and similar items. Such trading has mushroomed in the past few years through the establishment of the Nixon-Kissinger policy of détente with the Soviet Union.

The DSB report, prepared at the request of senior Pentagon officials and written by a task force headed by J. Fred Bucy, vice-president of Texas Instruments, argues that national and international arrangements for controlling east-west trade have not been effective in preventing strategic technologies from leaking eastwards through commercial channels. It therefore argues for a new approach, including some harsh sanctions to prevent such transactions.

Trade between the United States and communist countries has been controlled since the beginning of the cold war by a mechanism operated by an office in the Department of Commerce called the Office of Export Administration (OEA). A company wishing to export a strategically sensitive product or technology, as defined in a document known as the Commodity Control List, must apply to OEA for a licence.

The application is reviewed by an inter-agency committee composed of representatives from the Departments of State, Defense, Commerce, the Energy Research and Development Administration, and the CIA. Approval is given only to those transactions deemed unlikely to improve the Soviet Union's military capabilities.

A measure of western conformity in such matters is provided by an arrangement known as the Consultative Group Coordinating Committee (CoCom), consisting of representatives from NATO member countries, excluding Iceland but with the addition of Japan. CoCom maintains a list of restricted products similar to the US Control List, and its members have informally agreed to follow similar procedures in licensing exports to communist countries.

Although both the US Commodity Control List and the CoCom list have recently been brought up to date—during the height of the cold war they were said to contain such strategic items as brassieres and wigs—the DSB task force suggests that they still contain many products of little military value. Instead of wasting time on such items, the task force suggests that the list should be drastically shortened and more effort should be put into curtailing the transfer of vital design and manufacturing knowledge. Particularly close attention, it indicates, should be paid to deals involving the transfer of turnkey factories, products requiring sophisticated maintenance and operating information, and items resulting from a revolutionary technological advance.

The report argues that controls have broken down in recent years because "CoCom members have perceived less of a need to maintain strict controls while the opportunity for individual gain through the sale of technology to communist countries has increased. As a result, strategic technology has been transferred to communist nations through CoCom-sanctioned exceptions, ambiguous interpretations of lists, and, perhaps, conscious violation of CoCom agreements". The task force therefore suggests that the United States should try to bully CoCom countries into adhering to export control agreements by imposing "a sanction on any CoCom country that fails to control a specific technology, by restricting the flow of know-how in that technology to the offending country".

Another reason why controls have broken down, the task force argues, is

that firms in some countries which import US technology promptly re-export it to the Soviet Union, in violation of international agreements. Though the United States already attempts to stop such transactions by blacklisting firms thought to be engaging in such practices, the task force argues that some strong-arm tactics are in order here, too. "A nation that allows strategic technology to be passed on to a communist country should be restricted from receiving further technology of US origin", it states.

Moreover, the task force suggests that the United States "should release to non-allied, non-communist countries only the technology we would be willing to transfer to communist countries directly". In that regard, the task force states that "of particular concern is the acquisition of high technology know-how by nations of the middle east, and the assimilation of know-how by nations of Western Europe that are not members of CoCom—principally Switzerland, Sweden and Austria".

Though the bulk of the report deals with transfer of strategic technology through commercial channels, it also notes that recent scientific exchange agreements between the United States and the Soviet Union have greatly increased the movement back and forth of scientists between those two countries. Such agreements, Bucy notes in a covering letter to the report, are potentially "an area of concern".

The task force, finally, comes up with a recommendation that the Department of Defence should conduct a "comprehensive study of active mechanisms for transferring technology that are beyond the normal scrutiny of export control administration", and that it should develop recommendations for "monitoring and controlling them". Among such mechanisms, the task force lists "the use of US citizens as consultants for key technologies by communist countries", "the participation of US citizens as principals in firms established outside the US and engaged in transferring embargoed technology and products to communist nations", and "the training of citizens from communist countries at the more significant laboratories of US technical institutes and universities".

The task force report was approved for publication by Malcolm Currie, Director of Defense Research and Engineering, which suggests that it at least has his general approval. An internal task force has now been established in the Pentagon to evaluate the report and to recommend if, and how, its recommendations should be implemented. □

USSR

Conditions on educational success

The Soviet Union's Five-Year Plan for 1976-1980 will make increasing demands upon the higher educational system. Vera Rich looks at what new degree regulations being introduced could mean for Soviet science

By 1980 the Soviet economy will require 9.6 million "specialists" and 11 million persons with higher educational qualifications in order to attain the envisaged levels of economic growth and technological expansion. Hardly surprising, then, that considerable attention is being paid to problems of streamlining and improving the Soviet education system, particularly in the higher echelons; but a review of the method of awarding higher degrees is producing changes which can only be regarded as worrying.

The degrees of Candidate of Science (roughly equivalent to PhD) and Doctor of Science are not awarded by the Universities, but the Ministry of Education; Universities and Research Institutes make the necessary recommendations, but the confirmation is by no means automatic. The body which assesses the recommendations and awards degrees is the Higher Qualifications Commission (*Vyshshaya Attestatsionnaya Kommissiya*, VAK) of the Ministry.

On January 1 of this year, however, new regulations came into force, introducing a major reorganisation in both the structure of VAK and its operation which must, inevitably, reflect on the whole structure of Soviet research and the academic establishment. According to Professor V. G. Kirillov-Ugryumov, the Chairman of VAK, in a recent *Pravda* interview, these changes are not merely of an administrative nature; they also have a qualitative significance. The status and "potentialities" of VAK

have been extended. Requirements have been raised regarding the level of qualifications and, consequently, on entry into a scientific career.

In its reorganised form, the administration of VAK will be in the hands of a Plenum, a Presidium and a "College", in whose work leading scientists and specialists will participate. There are to be 34 councils of experts dealing with the principal lines of research, and also some 500 "specialised councils" for assessing degree theses, 150 of these councils being entitled to deal with Doctoral degrees. The Presidium and "College" of VAK have been operating since May 1975, and the first session of the Plenum met last July, but the establishment of the councils is not yet complete, although it is hoped to have them all fully organised by June of this year.

Regarding the "qualitative" changes, the new regulations, says Professor Kirillov-Ugryumov, will demand a higher standard in the theses submitted, and the postulants themselves will also have to meet certain more stringent requirements. The new regulations require that they should "combine a profound professional knowledge with a mastery of Marxist-Leninist theory, and with the convictions of an active builder of communist society".

Courses in political theory and participation in practical "community activity" have always formed a *de facto* compulsory part of Soviet higher education, but the student body and even the staff have tended to regard them as a necessary chore demanding little more than their physical presence. Hitherto the Soviet educational system, although Party-orientated, has permitted a certain number of non-Party scientists to rise to considerable academic heights (Academician Sakharov being the most notable example). The new stress on the political outlook

for higher degree postulants, in which their "community and political activity" as well as their scientific knowledge is taken into consideration, and the presence of Party and trade-union representatives on the "specialised councils", suggest that these days may be over, and that the non-Party element in science will gradually be phased out.

Significant, too, is the fact that almost all Professor Kirillov-Ugryumov's comments on the "distinguishing features" of the new regulations deal with these political aspects; on the academic side he notes merely that all exemptions from the examinations for the degree of Candidate and other "weaknesses" of the existing system will be eliminated, and that a higher standard will be demanded from the "official opponents" in the formal defence of doctoral theses.

Although the new regulations had not been published at the time of their introduction (a booklet of the full text is promised), their content and implications were well-known in Soviet academic circles some months in advance. As early as August 1975, reports from dissident sources were affirming that, under the new system, from 1985 onwards no non-Party member would be able to hold a major academic or scientific post, so that students wishing for a successful career in science would be well-advised to take steps to enter the Party ranks (via the Komsomols) at their earliest opportunity.

Politically speaking, the new emphasis on *partiinost'* does make a certain amount of sense—it was precisely the participation by the non-Party scientists that gave the dissident movement its real strength and significance. Scientifically speaking, however, while the new regulations may, in Professor Kirillov-Ugryumov's words, reflect a "qualitative" change, historical parallels would suggest that it is one which, in the long run, could be detrimental to Soviet science as a whole. □

ITALY

Trieste still troubled

Gillian Boucher reports from Trieste on the threat facing an international institution supported by funds from diverse sources

THE International Centre for Theoretical Physics at Trieste is threatened with a serious cut in funds which, if not compensated by money from other sources, could result in its closure.

What is in jeopardy is the contribution from the United Nations Development Programme (UNDP), which was to have provided nearly one-fifth of the 1976 budget of \$1.2 million. UNDP provides funds for specific scientific activities: the regular programmes on solid state physics and applied mathematics. Since an irreducible 35% of the total budget goes on overheads, cutting out UNDP support would mean an enormous reduction in the scientific

work of the centre.

Disaster almost struck just before Christmas when the centre received a cable from UNDP announcing that no money would be available for the course (then due to start in three weeks' time) on the interaction of radiation with condensed matter, and that the sum for the 1976 mathematics course would be halved. Fortunately the centre had in writing UNDP's previous promise to support these courses, and managed to persuade UNDP that it was impossible to cancel the solid state course. Funding for the course was

restored 17 days before it began, but the cuts for next autumn's mathematics course remain.

More serious is the likelihood that UNDP funds will soon dry up altogether. UNDP has been suffering badly from inflation and from the failure of some countries, notably the USA, to pay up. Its projected shortfall for 1976 of \$100 million means cutting back on many projects, and Trieste is a likely victim. Trieste is also likely to be hurt by the growing proportion of UNDP funds being assigned to individual nations rather than international projects.

The centre has always found living with UNDP a precarious business: meetings and courses have to be planned two years ahead while UNDP commits itself for only a year at a time. The Swedish International Development Authority, which provides the bulk of the funds to support the centre's Associates (scientists from less developed countries who come to do research at the centre three times in five years for up to three months at a time), also votes funds annually. More stable are funds from the Italian government (currently providing \$350,000 a year) and the International Atomic Energy Agency (IAEA) (\$225,000 a year), which votes funds on a two-yearly basis. To ease planning headaches and reduce the "rampant feeling of uncertainty" among the centre's small technical staff, an *ad hoc* consultative committee did in fact report to IAEA last summer that the unstable UNDP contribution should be taken over by the centre's stable sources of income.

With luck that is what will now happen. IAEA and UNESCO have recently expressed a wish to compen-

sate the centre if UNDP funds dry up, and are proposing to increase their funding to \$450,000 and \$300,000 respectively. Two potential problems seem to have been overcome. The first was that the IAEA contribution has been no greater than that of UNESCO since 1970, the year in which UNESCO first became a major source of funds. IAEA has however now agreed that its contribution need not be fixed at the UNESCO level, and indeed that with UNESCO's current impoverishment such a rule would be absurd. The other difficulty was that UNESCO's policy has been to fund a project for no more than 10 years, after which other sources of income (primarily national governments) are supposed to have been found. But there is now hope that the rule will change, as the committee studying it recently reported to UNESCO that it is outdated, a relic of the era of centres set up by regional bodies. Certainly it would be short-sighted in the extreme to apply the rule to a centre used by over 50 nations, most of them poor.

There should thus be no fundamental difficulty. The centre's well-being will depend upon the attitudes of those attending the imminent executive board meetings of IAEA and UNESCO and their general conferences in September and November of this year. At Trieste there are worries particularly about Britain's representative at UNESCO, Sir Harold Thompson, who is likely to repeat his view that Trieste is eating up too large a slice of UNESCO's \$1 million basic science cake, and may sway some of the Commonwealth countries. Ironically, at the same time that British scientists have provided the strongest support for Trieste and the backbone of its teach-

ing, the British government, through the international agencies, has caused it great worries.

If the proposed increases are approved by IAEA and UNESCO the centre will be slightly better off than at present but will of course have to exist at this level of funding through the next four years' inflation. Next year is in any case bound to be a messy one because the present uncertainties about money make planning almost impossible. But this year's programme is the largest ever and it is hoped at least to maintain the present level of activity: three 3-month courses and around 1,000 scientists passing through each year. New ideas are being tried; this summer there is a science teaching programme designed in particular for those scientists whose backgrounds are so weak that they cannot benefit from the other activities of the centre. The emphasis of the centre is on science at all cost; two of the three senior administrators as well as several secretaries have recently been made redundant, with the inevitable result of reduced efficiency and some discontent among the remaining staff.

If the UNDP funds are not replaced by those from IAEA and UNESCO, the centre's director Abdus Salem would prefer it to close rather than to continue with a grotesquely reduced programme. Closure would be a tragedy. The centre educates, stimulates and boosts the morale of thousands of physicists from less developed countries, providing them with the sort of contact with their peers which any American or European scientist takes for granted. And it is really very cheap: its running costs are the equivalent of less than 1% of Britain's SRC budget. □

JAPAN

China energy link weakens

JAPAN's energy policy still accords an essential role to oil, even though the crisis precipitated in 1973 showed it to be the most vulnerable of the industrialised countries because of its great dependence on oil imports. Like those countries, however, Japan is now having to cope with an emerging glut of oil, and in the last couple of months relations with one comparatively new supplier in particular appear to have changed.

The supplier is the People's Republic of China, with which the level of Japan's two-way trade last year reached a record \$3,800 millions. The two countries began their oil trade in 1973, and last year oil produced almost half of China's export earnings from Japan.

In January there were reports,

viewed at the time rather sceptically by Japan's refiners and electricity companies, that China might buy over 60% more steel from Japan in return for Japan increasing its purchases of Chinese oil from 8 million tonnes last year to 10 million in 1976.

The following month, however, before any 1976 agreement was concluded, China advised Japan that its February shipment would be halved. The same was expected for March as well, and one theory had it that supplies were being diverted to North Korea. When the first contracts were finally agreed in early March, the trend was confirmed: one of Japan's two major importing consortia agreed a 12½% cut. The other principal buyer has since contracted to take a

30% reduction. Japan's total imports of Chinese oil are thus unlikely to exceed 6.1 million tonnes, although there are options which, if exercised, would boost the total to 1975 levels.

It now appears that the February reductions merely anticipated the smaller contracts, and that the reductions themselves simply reflect Japan's oil needs at the present time (with the fact that China's oil is more expensive and less easily processed also playing a part). China may still be hoping to cash in on any upturn in Japanese demand, and might then be looking to offer longer-term oil contracts in return for the Japanese steel it now seems to want and which Japan's steel companies (shareholders in the oil importing consortia) are keen to sell. For the moment it is China's political upheavals which may preclude immediate progress on this.

IN BRIEF

Kissinger on sea law

Dr Henry Kissinger has urged the United Nations Law of the Sea Conference, now in the middle of its latest session in New York, to reach an early agreement, and has made suggestions to this end. Agreement was near on issues like the 200-mile economic zone and a 12-mile territorial limit, and on the continental shelf and protection of the marine environment; but, he said, the critical issues still to be settled concerned exploitation of deep sea beds, on which the USA's proposals were for an international sea bed resource authority, and arrangements for compulsory and impartial settlement of disputes. There was also the matter of marine research within the 200-mile zone. (The US Senate passed a bill at the end of last month to establish such a zone off

its coasts; it gives fishing priority to Americans and requires foreign vessels to obtain permits.) Dr Kissinger said the various issues were linked: failure to agree on them could eventually lead to war.

Energy consumption down

Figures released in the last fortnight confirm that total energy consumption in both the United States and the United Kingdom has been falling, and for essentially the same reasons. Apart from a stimulated interest in energy conservation and the benefits of a comparatively mild winter, the chief factors responsible for the reduction in both countries have been higher energy prices and the industrial recession. The US figures reveal a 2.5% drop last year (4.8% over the last two years),

while Britain experienced a 4.5% fall in the winter months of November, December and January. Oil consumption was down 5% in Europe and 1% in North America last year.

Middle East nuclear weapons

A report that Israel has an arsenal of 13 locally-made atomic bombs, decried in the Israeli press as speculation and part of a propaganda campaign by US officials favouring American arms supplies to Arab countries, provoked a response from Egypt's President Sadat last week on his visit to Italy as part of a European tour. He said he did not know if the rumours were true, but warned that Israel would have to face the consequences if it used nuclear weapons in the Middle East.

As money to support scientific research becomes scarce, it is right that we should scrutinise spending on what may possibly be fringe activities. Today a great deal which might go to pay salaries or to buy equipment is spent on organising meetings and conferences, and in paying the travelling and other expenses of those who attend. This can only be right if the scientists who attend these conferences do better research as a result. No one seems to have studied the "cost-effectiveness" of the scientific meeting.

Before the last war most of us seldom expected a grant and we went to conferences in our own countries or abroad at our own expense; if a small sum of money was available, it was received with gratitude and divided among a group of colleagues who gladly made up the balance out of their own pockets. Now most scientists expect the fullest public support, and there is even trade union pressure to insist that all shall receive the maximum government rate for the country visited, even if this is excessive, and even if poorer colleagues are denied a share of their costs. The total sum involved is substantial.

I would not deny that some conferences are fully justified; I think that they can be easily identified. Thus I personally gained a great deal from a Ciba Foundation Guest Meeting convened in January 1966 by Dr T. A. Quilliam of University College, London, on the apparently restricted subject of "The Mole; its adaptation to an underground environment". Some of us, all actively working with this animal, met for two days of

papers and discussions. Most of the work described had not yet been published. Everyone was interested in everything that was said, and all could enter fully into the extended discussions.

Conferring doubt**KENNETH MELLANBY**

Another equally valuable, though slightly larger meeting was an Advanced Study Institute, sponsored by NATO, and organised by my colleague Dr N. W. Moore at Monks Wood Experimental Station in July 1965. Its subject was "Pesticides in the environment and their effects on wildlife". About 75 scientists, including the majority of those from all parts of the world who were actively researching in this field, met for a fortnight to compare notes. Most of

the papers dealt with unpublished material. Everyone was deeply concerned in the subject and able to contribute something useful to the discussion.

Thus it would appear that small, highly specialised meetings can be successful, and helpful to research. I fear that the same cannot be said for the enormous international gatherings arranged by many learned societies. Here few papers contain anything new. Most of the speakers read a paper as a condition of receiving a grant. Many of those supported by public funds are absent from most of the sessions. Sometimes it is an opportunity for younger scientists to see and hear some of the elderly savants who may already possess legendary reputations, but unfortunately they often discover that their idols have tongues of clay.

It may be admitted that the public sessions of large conferences are unproductive, but it is suggested that the informal contacts, in the bar and elsewhere, justify the expense. I cannot confirm this. I have asked many colleagues whether they can honestly say that such contacts have ever had a major and productive effect on their research. So far no one has given me an example. Contacts between working scientists are undoubtedly important, but large meetings with their social distractions may be the worst places for them to take place. The distractions may be fun, but why should they be enjoyed at the taxpayer's expense? The money could probably be better spent sending young and productive scientists on visits to selected laboratories.

correspondence

Incubator contamination

SIR,—Tissue cultures usually require a CO₂-in-air incubator to maintain the proper pH in the medium. When chemicals are introduced into the culture to test for their carcinogenic or mutagenic activity, potentially hazardous materials might be volatilised and released through the incubator vent.

Air from an incubator (37 °C) containing four large covered dishes, each of which held three smaller covered dishes containing about 50 µg of safrole in 8 ml of medium, was drawn through a bed of activated charcoal. The charcoal was treated with CS₂, and the eluate was analysed by gas chromatography. The concentration of safrole in the gases vented from the incubator was about 2×10^{-2} parts per 10⁶.

Concentrations of such magnitude may prove to be insignificant, but investigators should be aware of this possible source of contamination. A partial solution to potential exposure of personnel may be achieved by connecting the incubator vent to an exhaust system (for example, an operating chemical fume hood) by large-bore tubing. This expedient will, however, neither prevent the release of potentially contaminated air into the laboratory each time the incubator is opened, nor eliminate the possibility of transmission of volatilised material from one dish to another within the incubator.

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Angling right

SIR,—Kenneth Mellanby (March 4, page 7) is right to claim that anglers are responsible for the continued existence of the large quantity and good quality of freshwater fish in parts of the United Kingdom, particularly in England where the need to pollute is greatest.

In earlier and cleaner times, when fish were threatened by over-harvesting, 74 Angling Societies in London were the principal supporters of the Mundella Bill for the Preservation of Non-migrating Fish in England and Wales. It became law in 1878 (though not before jealous rows between the larger of the Societies and after fierce debates on whether or not to include a close season for the migrating species), and for the first time curbed the netting and marketing of "white fish", which

by that time had reached wholesale proportions in the effort to feed fresh protein to a growing population.

Once refrigeration, Argentine beef and New Zealand lamb became available the pressure on freshwater food eased dramatically, and it is rare to meet anyone in this country who enjoys aromatically baked pike, grilled perch or the carp, once the favourite of the abbots. All three species are the equal of the salmon and infinitely superior to the pathetic off-fed restaurant trout.

Today, as Mellanby emphasises, the danger is pollution in all its many and sometimes subtle guises, and it is curious how long it was before anglers organised themselves in a co-operative to spread the risk of the cost of bringing a Common Law action against interference of any kind with an established fishery. John Eastwood founded the Anglers Cooperative Association in 1948, and it has since won costs and compensation in almost a thousand cases of pollution, losing one on a technicality.

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Science indicators

SIR,—It is often asserted that science ranks with politics at the bottom end of public esteem. But the survey conducted for the National Science Foundation in the US (March 18, page 184) discerned a remarkably favourable attitude toward science and technology among Americans. The survey found that 75% of the American public believes that science and technology have changed life for the better; a similar survey conducted in 1972 discovered that only 70% then held that belief. In addition, scientists and engineers were ranked second and third respectively in terms of occupational prestige—below doctors, but above ministers of religion. Businessmen ranked lowest.

As for the impact of science and technology, there seems to be a certain amount of ambivalence. Some 57% of the respondents said they think that science and technology have done more good than harm, while 31% said they believe the beneficial and harmful impacts are evenly balanced. Only 2% believe that science and technology have done more harm than good. By far the most frequently cited benefit was improvements in medical care.

When asked whether social control

over science and technology should be increased, 28% of the respondents replied that it should, while 46% said they believe the degree of control is about right.

The results of the survey also conflict sharply with the popular belief that young people are particularly turned off by science and technology—the attitudes of people under thirty differed little from those of elders.

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Mass and weight

SIR,—The introduction of the SI system of units presented a great opportunity to clarify the difference between the concepts of mass and weight. It is thus disappointing that the use of the word kilogram has already in common parlance become associated with weight rather than mass. This is illustrated in the jingle put out by the British Metrication Board which you quote in an example in Competition 6 (March 18, page 188).

Although in most cases weighing is merely a convenient way of determining mass and "weights" used with balance-type instruments are calibrated in mass units, it would have been preferable, for example, to express the contents of a jar of jam as net mass 453 g rather than net weight, as has become the custom. The widespread television coverage of space flights has well illustrated the concept of weightlessness and it should no longer be difficult even for children to appreciate the distinction between mass and weight.

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The JPU in modern science

SIR,—D. Fraser is to be congratulated for his valuable concept of the JPU (Just Publishable Unit) presented in *Nature* (Correspondence, March 11). A more conservative measurement, however, is the JXPU (Just Xeroxable Published Unit), which may be defined as the smallest amount of published information normally considered worthy of xeroxing. An example being D. Fraser's correspondence.

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news and views

REPORTS of unusual aerial phenomena always attract great interest, are surrounded by folklore and superstition and irritate scientists who find them hard to explain. By far the most common such reports concern luminous spherical or ellipsoidal objects which appear without warning, often move through the air erratically, and disappear without trace, sometimes explosively. Each year, hundreds of eye witness accounts of such events are reported to local newspapers, the police and even military authorities. Occasionally the accounts are published in scientific books and journals, and several have appeared in *Nature* in the past few years. When the events occur during adverse weather conditions they are often called ball lightning. The invention of a precise name is somewhat misleading because it suggests that these phenomena are well understood. They are not. Indeed, so puzzling and bizarre are the properties attributed to these luminous balls that many scientists have preferred to deny their existence.

For the theoretical physicist, the hardest thing to swallow is that the balls are often said to be encountered inside closed, or nearly closed, structures. One spectacular report appeared a few years ago of a luminous sphere moving down the inside of an all-metal aircraft in flight. More frequently, witnesses claim to observe ball lightning inside their own homes.

One particularly alarming account of such an event is described by M. Stenhoff of Royal Holloway College, London University, in this week's issue of *Nature* (page 596). The case is particularly intriguing because the single witness, a lady living in the English Midlands, was able to produce material evidence in support of her story. During the course of her unnerving experience, the witness claims she was struck full in the body by a blue sphere of light, which totally disintegrated her clothing at the centre of impact. The dress she wore has a hole several centimetres across. Surprisingly, she sustained little physical injury, complaining only of a burning sensation in her wedding ring when she instinctively brushed the object away.

Taken at face value, this report highlights the hazards surrounding the present lack of understanding about these phenomena. It is not at all clear, for example, that conventional light-

Ball lightning

from P. C. W. Davies

ning precautions are of any use against such things, or indeed that ball lightning actually has any direct connection with ordinary lightning. None of the several theories which has been proposed in recent years accounts for the properties of the luminous spheres completely satisfactorily. The most difficult part is to explain how they can remain coherent, stable and luminous for durations up to, apparently, some minutes. There is also the peculiar kinematic behaviour—in many cases the balls are said to move upwind, and even trail along beside aircraft travelling at several hundred m.p.h. through the air. Electrical explanations based on ionic recombination fail to explain the long lifetimes. Various plasma theories have been published, but run into problems of stability. Even nuclear theories have been tried. They would predict that the Midlands lady should be suffer-

ing from radiation sickness. Perhaps the most imaginative suggestion of all is that micrometeorites of antimatter are responsible!

One of the most promising theories is due to the Russian physicist Peter Kapitsa, who has proposed a model based on the existence of UHF electromagnetic waves produced by an ordinary lightning strike. A standing wave pattern could then be created under some circumstances, which might then ionise the air in the region of an antinode, thus producing a luminous patch which could be sustained in energy by the external field. This theory certainly accounts for many of the enigmatic properties of the luminous balls, but is not completely convincing. It seems likely that some nonlinear, coherent electromagnetic process which is still not understood is regularly taking place in the atmosphere.

As always with transient aerial phenomena, scientific progress is hampered by lack of precise observational data. Accounts often reflect the beliefs and perceptual distortions of the witnesses. In addition, the usual unreliability of the human memory for accurately recalling details of speeds, sizes, durations and so on makes any theorising based on random narrative evidence from the general public particularly risky.

The present unsatisfactory situation would be greatly improved if the aura of mystery and superstition surrounding unusual aerial events were dispelled. Good, detailed eye-witness reports of luminous balls are frequently made by competent observers such as airline pilots, but are rarely passed onto scientists. Instead many of them find their way into military files, where they are shrouded in a ridiculous secrecy. (Incredibly, the British Ministry of Defence continues to deny scientists access to their accounts of these events.) With proper cooperation between scientists and the public, particularly the local press, and the civil aircraft authorities, it would be possible to follow up ball lightning reports rapidly, enabling tests for radioactivity and so forth to be carried out. One sensible improvement would be for the Meteorological Office to draw the public's attention to the existence of this potential hazard, particularly during stormy weather, and request detailed reports. □

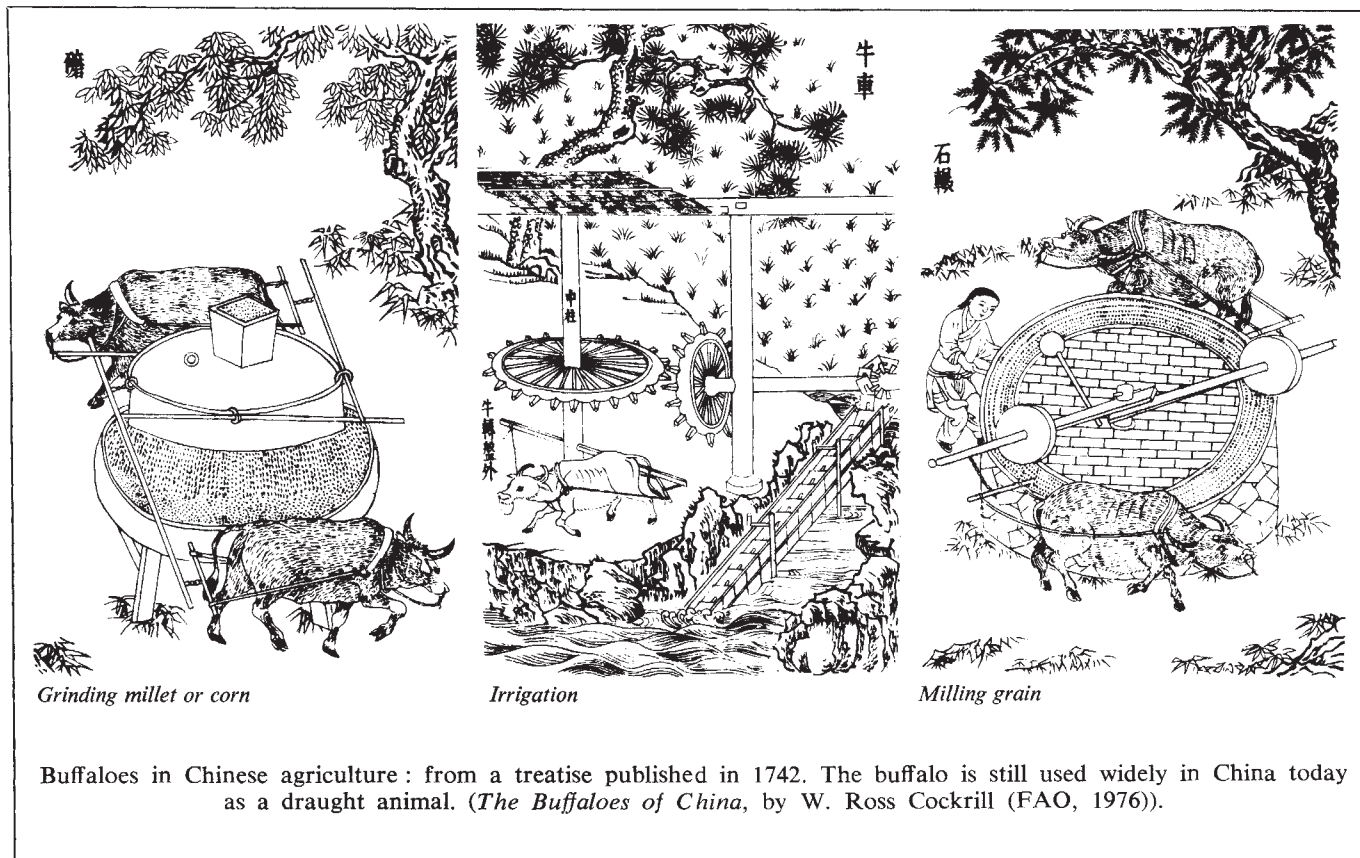


A hundred years ago

A CORRESPONDENT, Mr. F. Green, writing from Cannes, France, states that on the 8th instant, for the first time this year, he heard the Cuckoo in a valley amongst mountains sixteen miles to the westward of that place. The first time last year that he heard it in the same neighbourhood was on the 10th of April.

ON April 2 at 5.55 a.m., an earthquake was felt at Berne. Two movements took place from east to west. The duration at was two seconds; doors were opened, and church bells were rung by the shocks. In Neufchatel a strong detonation was heard; the oscillation was very strong in the lowest part of the city, and clocks struck the hour before the appointed time. Persons who were in the streets declared that warm wind was blowing for some seconds. A few hours afterwards a rain-spout occurred near Mainz, in Rhenish Hesse. A number of houses were struck by a thunderbolt and ignited, many others were flooded by the water falling from the mountains, and people drowned by an instantaneous flood.

from *Nature*, 13, April 13, 475; 1876.



Order and spacing of ribosomal RNA genes

from Benjamin Lewin

THE first system to be defined in which a large precursor RNA is processed to much smaller mature molecules was that of rRNA synthesis in eukaryotic cells; as early as 1966, Greenberg and Penman (*J. molec. Biol.*, **21**, 527-535) reported that a label in nucleolar RNA first enters a 45S species in HeLa cells and only later is found in 28S and 18S rRNAs. Since then it has become apparent that the 45S RNA contains the sequences of both the 28S and 18S RNA molecules, which together occupy about 7,500 nucleotides of its total length of 14,000 bases.

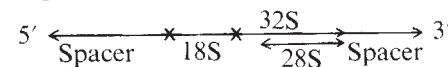
In bacteria the situation at first appeared to be different, with the 16S and 23S rRNAs each apparently transcribed independently, although their genes were located adjacent on the chromosome. But the isolation of an *E. coli* strain lacking a processing enzyme allowed Nikolaev, Silengo and Schlessinger (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3361-3365; 1973) and Dunn and Studier (*ibid.*, 3296-3300) to isolate a 30S precursor molecule that contains both sequences.

How are the mature rRNA sequences organised within the precursor? In bacteria the sequences 5' 16S-23S-3' is generally agreed on the basis of the relative sensitivity of 16S and 24S RNA synthesis to inhibition by rifampicin

(for example Doolittle and Pace, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1786-1790; 1971). In eukaryotes the evidence has been conflicting although up to now it was generally held to favour the reverse order. In particular the identification of distinct secondary structure of the various rRNAs and their precursors in the electron microscope and the determination of polarity by digestion with a 3' exonuclease, suggested that the 5' end of the 28S molecule coincides with the 5' end of the 45S precursor (Wellauer and Dawid, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2327-2831; 1973). This seemed to be the definitive answer and a similar organisation for *Xenopus* 40S precursor was suggested by the same authors (*J. molec. Biol.*, **89**, 379-396; 1974).

But two recent experiments using different techniques suggest the opposite conclusion. Hackett and Sauerbier (*J. molec. Biol.*, **91**, 235-256; 1975) inhibited RNA synthesis in L cells by ultraviolet irradiation. The irradiation causes transcription to terminate at damaged sites in DNA; the farther a gene lies from its promotor, the more likely it becomes that a terminating event will occur before its transcription is completed. The pseudo-first order loss of 45S RNA as a function of ultraviolet dose confirmed that each

gene for the precursor represents an individual transcription unit with its own promotor. The target size of the 18S cistron suggested by these experiments was about 4,000 nucleotides, just twice the length of the 18S RNA molecule. This suggests that a spacer of 2,000 nucleotides lies at the 5' end of the 45S precursor, followed by the 2,000 nucleotides of the 18S sequence. The target size of the 28S RNA was 11,000 nucleotides; since the molecule is 5,500 nucleotides long, it must begin at a point 15,000 nucleotides after the end of the 18S RNA. This leaves a distance of 3,000 nucleotides at the 3' end of the 45S RNA, which must be part of the discarded sequences. The target size of the 32S precursor, the immediate precursor of 28S RNA, is the same as that of the 28S molecule; this suggests that the 32S precursor is adjacent to the 18S sequence, so that the 45S RNA has the structure:



Another approach to analysing sequence organisation has been taken by Liau and Hurlbert (*J. molec. Biol.*, **98**, 321-332; 1975). The mature 28S and 18S RNA molecules are methylated on the 45S precursor, when only these sequences gain the methyl groups. The

relative amounts of methylation of the two mature molecules are different, so that methylation could be used to identify the parts of the 45S precursor carrying these sequences. The experimental problem is to obtain identified fragments of the 45S molecule. Liao and Hurlbert generated suitable conditions by incubating Novikoff rat tumour nucleoli in the absence of nucleotides; this prevents elongation of polynucleotide chains, generating a series of incomplete precursor molecules of varying lengths starting from the 5' end, which are methylated *in vitro*. The 18S methylation pattern is found in the shorter transcriptions and the 28S pattern is seen in the longer molecules.

One of the most satisfying conclusions to be drawn is that the same relative organisation of mature rRNA sequences within the precursor is seen in bacterial and eukaryotic systems. The smaller molecule precedes the larger, although more extensive spacer sequences have evolved in the eukaryotic systems. It seems clear that the relative locations of the 18S and 28S RNA sequences seen in the HeLa and *Xenopus* precursors by electron microscopy are indeed correct; but the polarity is reversed. The explanation seems to lie in the use of the exonuclease to determine the polarity of the RNA; clearly in the system used, the 5' rather than the 3' end must have been digested, perhaps by some contaminating enzyme.

What is the function of the spacer regions in the common precursor? Little information is available on the extensive spacers of the eukaryotic precursor, but the striking observation that a tRNA gene lies between the 16S and 23S sequences in an *E. coli* unit has been reported by Lund *et al.* (*Cell*, 7, 165-177; 1976). The *E. coli* genome possesses several copies of the genes for rRNA, organised in transcription units that display the sequence 16S-23S-5S RNA (with the assignment of the 5S RNA to the end of the unit being less certain than the tandem arrangement of the 16S and 23S sequences). The transducing phage λ rif¹⁸ carries one of the sets of the genes for 16S and 23S RNA, as well as genes for some RNA polymerase subunits, ribosomal proteins, and a protein synthesis factor. When cells are infected with this phage, the synthesis of two small RNA species is greatly stimulated: one is 5S RNA and the other is tRNA₂^{Glu}. Where is the tRNA gene located? By showing that a radioactively labelled tRNA₂^{Glu} molecule could hybridise to a phage DNA deleted for the 23S sequence, but could not hybridise to a phage DNA where the deletion extended from the 23S sequence into the 16S sequence, they

The Red Queen lives

from Leigh Van Valen

WHEN the Red Queen heard of her reported dethronement (Hallam, *Nature*, 259, 12-13; 1976), she smiled from her throne and said, "As Mark Twain remarked on a report of his death, I find the report somewhat exaggerated".

One side of a controversy is often convincing until the other side appears. Hallam primarily based his conclusion on two recent papers of Salthe and Sepkoski; my response to these has not yet been published and will be part of a broader discussion. For now I summarise my comments on the points Hallam discussed.

There are several possible hypotheses to explain the law of constant extinction, which produces a linearity of the survivorship curves of adaptively similar groups on a semilog plot. One is the Red Queen's Hypothesis, that the sum of absolute fitnesses in a community is constant. Another, used by Raup *et al.* (*J. Geol.*, 81, [for 1973], 525-543; 1974) in their simulations and which I call the Field-of-Bullets Hypothesis, is that the probability of extinction is constant for all groups at all times. This hypothesis is unlikely (Van Valen, *Evol. Theory*, 1, 1-30; 1973; *Nature*, 257, 515-516; 1975). A third hypothesis, which Salthe (*Paleobiology*, 1, 356-358; 1975) proposed and which may be called the Hypothesis of Triviality, is that any reasonable pattern of causation leads to near linearity and so no specific explanation is appropriate. There are several problems with the Hypothesis of Triviality, but it would seem to be disproved by the existence of a few systematically non-linear survivorship curves. That such deviations from linearity have known no possible particular explanations

(Van Valen, *Evol. Theory*, 1, 1-30; 1973) merely accentuates their reality. Simpson (*Tempo and Mode in Evolution*; 1944) used a phenomenon equivalent to part of this exceptional non-linearity as the basis for distinguishing what he called bradytely and horotely as more or less discrete evolutionary modes. Horotely is intimately related to linearity or survivorship curves.

Sepkoski (*Paleobiology*, 1, 343-355; 1975) in fact claims that most of the curves are non-linear and that the distribution of the lengths of stratigraphic units produces a bias that gives spurious linearity. However, the main result of this bias, when I apply my methods to his simulations, is a reduction in the proportion of bradytely taxa that are identified, rather than a qualitatively false conclusion of linearity and homogeneity. Sepkoski's analyses were mostly of situations more extreme than the ones I used and most of his results have little bearing on mine. I originally excluded some data from any analysis because of related inadequacies. There are many kinds of inaccuracies involved in my analysis, some as yet unpublished, but none known to me seriously affect the predominant linearity of the curves.

The Red Queen's Hypothesis is ecological and does not depend on the linearity of taxonomic survivorship curves which served as midwife. Her domain is less than universal and may be empty: the hypothetico-deductive method is weak, and the sort of facts from which the hypothesis may be deduced are as yet only suggestively available. But the Red Queen is rather flexible, as a runner should be, and her domain may yet include much of evolution.

were able to conclude that the gene for the tRNA must lie between the genes for the 16S and 23S RNAs (Yamamoto, Lindahl and Nomura, *Cell*, 7, 179-190; 1976). This location was confirmed by Wu and Davidson (*Proc. natn. Acad. Sci. U.S.A.*, 72, 4506-4510; 1975). in experiments utilising a new technique for visualising RNA-DNA duplex regions formed along a single strand of DNA.

An obvious question is whether this form of organisation is seen in all the 16S-23S transcription units of the bacterial genome. Lund *et al.* (*op. cit.*) examined the transducing phage ϕ 80rif^r, which also carries an rRNA transcription unit, and showed that it is homologous with the λ rif¹⁸ phage over most of the bacterial sequences

except for 310 base pairs between the 16S and 23S rRNA sequences. This means that the internal spacers are different in the two transcription units. When cells are infected by ϕ 80rif^r tRNA₁^{Ile} is synthesised, and Lund *et al.* have confirmed that its gene seems to lie within the spacer region. At least two of the rRNA transcription units of *E. coli* therefore possess a (different) tRNA sequence; whether the other (approximately 5) units also carry tRNA sequences remains to be established. What function in bacterial protein synthesis might be served by this coupling is not yet clear, although it seems to have the consequence that the tRNA coded with the rRNAs is synthesised in large amounts (both tRNA₂^{Glu} and tRNA₁^{Ile} are among the

more abundant of the *E. coli* tRNAs). The processing of the transcript of the complete unit releases four RNA species, in the order 16S, tRNA, 23S, 5S RNA; the enzyme that appears to undertake this reaction is ribonuclease III and by showing that a deletion phage lacking the end of the 16S sequence can still generate 23S and tRNA sequences, Yamamoto *et al.* (*op. cit.*) were able to infer that the complete precursor sequence is not required for proper processing. This supports the idea that processing can occur before transcription has been completed. □

Nuclear charge distributions

from P. E. Hodgson

NEW measurements of the cross section for the elastic scattering of electrons by lead have recently cleared up an outstanding discrepancy concerning the charge distribution in the interior of the nucleus. It has for a long time been uncertain whether there is a dip or a hump in the middle of ^{208}Pb , and it has now been established that there is a small hump.

At first sight this result seems surprising. We know that the protons in the nucleus repel each other by their electrostatic charges, and this might lead us to expect a dip in the charge distribution in the middle of the nucleus. We must however think of the protons as occupying shell model orbits, and the total charge distribution is found by adding the squared moduli of the wavefunctions of the protons in all the occupied orbits. Now the wavefunctions of the protons in S-orbits, with zero orbital angular momentum, have a finite value at the centre of the nucleus, whereas the wavefunctions of protons in all other orbits are zero at the centre. The value of the charge density at the centre of the nucleus thus depends quite critically on the number of protons in S-orbits. In lead the protons in the 3S orbits have the smallest binding energy, and so we might expect that these form a hump at the centre of the charge distribution, and this hump is indeed given by most calculations of the charge distribution of lead, for example those made using the Hartree-Fock theory.

Until recently, however, the experimental determinations of the charge distribution have tended to give a small central dip, and this could not be reconciled with the theoretical result. There was however some uncertainty about this because of the difficulty of obtain-

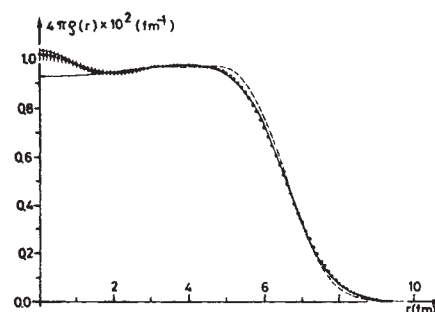
ing the charge distribution from the measurements of electron scattering.

Electrons only interact with the nucleus throughout their electromagnetic field, so if we scatter electrons by nuclei we should learn about the charge distribution of the nucleus. Unfortunately, although it is possible to calculate the scattering from a known charge distribution, it is not easy to reverse the calculation and obtain the charge distribution from the electron elastic scattering cross section. Some quite sophisticated techniques have been developed to do this, and the differences between them account for much of the uncertainty about the form of the charge distribution in the centre of the nucleus.

The simplest method is to assume a suitable analytic form for the charge distribution and then vary its parameters to optimise the fit to the electron elastic scattering cross section. One of the most popular forms is the Fermi function $[1 + \exp\{(r-R)/d\}]^{-1}$ which is uniform in the interior and falls rapidly to zero around the radial distance R , the steepness of fall depending on the parameter d . This was soon found to be insufficiently flexible, so it was multiplied by various polynomials such as $(1 + \alpha r^2)$, which makes it possible to introduce dips and humps in the interior.

Many analyses were made in this way, and it was often possible to obtain excellent fits to the scattering cross sections, but there was some doubt about the interpretation of the resulting charge distributions. It was realised that the scattering is most sensitive to the charge distribution in the 'knee' region at the surface of the nucleus, and the parameters are largely determined by the behaviour of the assumed charge distribution in this region, so that it is dangerous to assume that this gives us reliable information about the charge distribution in other regions such as the centre of the nucleus.

Much effort has therefore been devoted to the development of the so-called model-independent ways of determining the charge distribution from the scattering cross sections. Some of these use a series of concentric shells of charge, and adjust the densities of each, while others express the charge distribution as the sum of a series of terms each consisting of a suitable function of the radial distance, with different ranges and coefficients. Some important work of this type has been done by Friedrich and Lenz (*Nucl. Phys.*, **A183**, 532; 1972) and Friar and Negele (*Nucl. Phys.*, **A212**, 93; 1973), so that it is now possible to obtain the charge distribution without making any unduly restrictive assumptions about the form of the distribution.



The charge distribution of lead, as determined experimentally and by Hartree-Fock calculations. The experimental points correspond to the analysis of the Mainz data, the dashed line to the calculations of Vautherin and Brink using the Skyrme force, and the full line the result of an analysis using a three-parameter Fermi distribution that does not give an acceptable fit to the electron scattering data.

The new measurements that have recently been made to resolve the difficulty about the charge distribution in the centre of lead are due to the group of Professor Ehrenberg at the University of Mainz (Euteneur, Friedrich and Voegler, *Phys. Rev. Lett.*, **36**, 129; 1976). They scattered electrons of 120, 200 and 290 MeV from lead, and analysed the results using a very flexible expansion of the charge distribution in terms of a series of spherical Bessel functions of various ranges. Their results, as shown in the figure, provide definite evidence in favour of a hump in the centre of the nucleus, in accord with the theoretical calculations.

This work shows how accurately we now know the charge distribution of some nuclei. There still remain some uncertainties, especially in the region of low densities at the edge of the nucleus, and this will doubtless be the subject of further studies using electrons of higher energies. □

Role for histone H1 in chromatin condensation?

from Tom Barrett

THE lysine-rich histone H1 is the most unusual of the five major histone species found in eukaryotic chromatin. In contrast to the other histones which show a strict sequence conservation over a very long evolutionary period, the amino acid sequence of histone H1 varies to some extent (Rall and Cole, *J. biol. Chem.*, **246**, 7175; 1971). Its molecular weight (about 23,000 daltons) is greater than that of the other major histone species H2A, H2B, H3 and H4. It contains a very high proportion of

basic amino acids; approximately 25% of the molecule is composed of lysine residues. It is the most readily removed of all the histone species in spite of its very basic charge and can be washed off chromatin with 0.5 M NaCl. Immunological studies have shown that its antigenic determinants are more available in native chromatin than are those of the other histone species. In percentage terms its immunological availability at 9.6% is considerably greater than that of the next most available histone—H2B at 3.2% (Goldblatt and Berstin, *Biochemistry*, **14**, 1689; 1975). Crosslinking experiments performed on chromatin show that histone H1 does not readily form complexes with other histones but rather forms H1 oligomers (Thomas and Kornberg, *FEBS Lett.*, **58**, 353; 1975).

These differences are highlighted by the fact that histone H1 is not required for formation of the characteristic bead-like structures, called ν bodies or nucleosomes, found in native eukaryotic chromatin. Nucleosomes consist of a histone core made up of two each of the four histones H2A, H2B, H3 and H4 associated with approximately 200 base pairs of DNA. Recent neutron diffraction studies indicate that the DNA is wound in a superhelix around the outside of this histone core (Baldwin *et al.*, *Nature*, **253**, 245; 1975; Pardon *et al.*, *Nucl. Acids Res.*, **2**, 2163; 1975).

Chromatin condensation

Since histone H1 is not involved in the formation of the basic nucleosome structure of chromatin its function must be independent of that of the other histone species. There is strong evidence that histone H1 is responsible for chromatin condensation. In dilute buffer solutions chromatin forms a viscous gel which contracts to a maximum of 10% of its original volume as the ionic strength is increased. Increasing the ionic strength above a certain level causes the chromatin to expand again as the histone H1 begins to dissociate from it. The release of H1 from chromatin is governed by the effective ionic strength of the cationic component of the salt, 0.5 to 0.6 M being the critical concentration for release of H1 (Bradbury *et al.*, *Eur. J. Biochem.*, **57**, 97; 1975). These authors have made an extensive nuclear magnetic resonance (NMR) study of histone H1 both in free solution and in chromatin under various ionic conditions. In $^2\text{H}_2\text{O}$ virtually all the H1 resonance spectrum can be seen but the signals are broader than those of isolated H1. Addition of salt causes the resonances to broaden until a concentration of 0.4 M salt is reached when the spectrum begins to sharpen. When 0.5 to 0.55 M is reached

the H1 spectrum is fully developed with a marked increase in the intensity of the lysine resonances. Broadening of the peaks thus corresponds with the contraction of the chromatin gel. The contraction of the chromatin gel is not observed with H1-depleted chromatin. Spectra obtained for H1-DNA complexes are identical in nearly all respects with those obtained for chromatin under the same solvent conditions. The main difference is that the spectrum for the H1-DNA complex is not as broad as that of chromatin at low ionic strength and is evidence that there are more free H1 lysine residues in the complex than in chromatin. Probably one third of the lysine residues in the complex are free in these conditions in spite of the very high positive charge on the lysine molecule. The authors suggest that the addition of salt causes a structural change in H1 and/or reduces the repulsion between the DNA molecules, allowing them to approach each other more closely and bind the free end of the H1 molecule. In chromatin the other histone molecules are present and act to reduce the high charge on the DNA molecules.

Both the crosslinking experiments and the NMR resonance spectra indicate that in chromatin histone H1 is not associated with the other histones but is bound directly to the DNA. Structurally, histone H1 consists of an apolar-rich central region—residues 40 to 75—a short N-terminal end and a long C-terminal end rich in basic residues. The central apolar region is probably the site where the histone H1 molecules interact with one another. The long C-terminal end is the most basic region of the molecule and is probably the main site for the interaction of H1 with both free DNA and chromatin DNA (Bradbury *et al.*, *Eur. J. Biochem.*, **52**, 605; 1975). It has been reported recently that the aggregation of DNA molecules by histone H1 is much greater with superhelical DNA, irrespective of the sign of the superhelical turns, than with open relaxed DNA (Bottger *et al.*, *Nucl. Acids Res.*, **3**, 419; 1976). Since the DNA in the nucleosome appears to be supercoiled around the histone core this structure would favour the interaction of histone H1 with the DNA.

Whether or not histone H1 is associated with the DNA wrapped around the nucleosome proper or to the DNA linking adjacent nucleosomes is not certain. A recent paper by Varshavsky *et al.* (*Nucl. Acids Res.*, **3**, 477; 1976), however, lends support to the latter idea. They purified nucleosome monomers on sucrose gradients and on subsequent electrophoresis on polyacrylamide gels found two discrete types of nucleosome. The first contained all five histones associated with

200 base pairs of DNA, whereas the second lacked histone H1 and contained only 170 base pairs of DNA. They could also distinguish three types of dimer containing 0, 1 and 2 molecules of histone H1 respectively. They suggest that H1 is bound to a short (30 base pair) terminal stretch of nucleosomal DNA which can be removed by nuclease treatment, probably as a H1-DNA complex, without significantly disturbing the basic nucleosome structure.

Chromatin transcription

Histone H1 is absent or very much reduced in concentration in chromatin that is being actively transcribed. Calf thymus chromatin can be resolved into two fractions by chromatography on ETCHAM-cellulose. The first fraction to elute contains histone H1 whereas the second fraction is depleted in H1. This H1-depleted fraction is enriched in RNA polymerase binding sites and presumably corresponds to the active chromatin (Simpson, *Proc. natn. Acad. Sci., U.S.A.*, **71**, 2740; 1974). Using a different technique whereby Mg^{2+} is used to precipitate the condensed or inactive chromatin fraction, Gottesfeld *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2193; 1974) separated a soluble nucleoprotein fraction from rat liver chromatin. This fraction was enriched several-fold in transcribed DNA sequences and histone H1 was virtually absent. In the inactive, highly condensed chromatin of nucleated erythrocytes an additional histone species, histone H5 is present. In many respects H5 is similar to H1 and removal of both these histones from erythrocyte chromatin causes it to expand and resemble the uncondensed fraction of rat liver chromatin. Histone H1 seems to substitute equally well for H5 in the condensation of erythrocyte chromatin. It is probable that the highly condensed state of erythrocyte chromatin is not due solely to the presence of the erythrocyte-specific histone H5 but rather to a greater saturation of the H5 binding sites in erythrocyte chromatin. Reeck (*Arch. Biochem. Biophys.*, **172**, 117; 1976) could detect no H5-depleted regions analogous to the H1-depleted regions in calf thymus chromatin. In fact during the erythrocyte maturation process the concentration of H5 increases in parallel with a decrease in RNA synthesis (Billet and Hindley, *Eur. J. Biochem.*, **28**, 451; 1972).

There is good evidence that the other histone species are present in actively transcribed chromatin. The presence of nucleosomes on DNA does not seem to block its transcription. Lacy and Axel (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3978; 1975) isolated monomeric nucleo-

somes from rat liver chromatin and found that a further 46% of the DNA in the monomers could be digested. Analysis of the reassociation kinetics of the resistant DNA revealed that most, if not all, the genomic sequences were involved in this basic subunit structure and that virtually all the liver sequences transcribed into nuclear RNA were present in the nucleosomal DNA.

It appears that the histones act in two distinct ways to fold the DNA molecule in chromatin. First, the four histones H2A, H2B, H3, H4 form an octameric protein core around which the DNA is supercoiled. This results in a 7-fold contraction of the DNA but does not block transcription. Second, histone H1, when present, further condenses the nucleohistone and in so doing makes it unavailable for transcription. □

The illogic of conservation

from Peter D. Moore

It is difficult to be objective, or even rational, about conservation. This is especially true in situations where the conservationist is concerned with the continued survival of a species whose population has fallen to a critically low level. When the very existence of a species is threatened then the conservationist does have sound reason on his side, for extinction, and the depletion of the world's genetic resources which this represents, cannot be regarded dispassionately, yet in practice the attitudes of those who seek to guard the world's wildlife are often far more parochial and far less guided by logic than is normally supposed.

It is this criticism which John Gooders (*Brit. Birds*, 69, 16; 1976) has recently levelled at ornithologists. He believes that a disproportionate amount of concern and money is spent upon preserving isolated colonies of bird species in the peripheral areas of their global range simply because they are rare there and because the birdwatcher in the street has a consuming interest and is therefore willing to spend money on propping up rarities, even if they are common elsewhere in the world. Certainly it is not difficult to find examples of this kind of behaviour on the part of British birdwatchers. Gooders quotes such birds as the snowy owl (*Nyctea scandiaca*), osprey (*Pandion haliaetus*) and avocet (*Recurvirostra avosetta*) all of which are

Active Mercurian dynamo unnecessary

from Peter J. Smith

ACCORDING to measurements made recently by Mariner 10, the planet Mercury has a magnetic dipole moment of about 5×10^{22} gauss cm³ (5×10^{19} A m²), which is more than three orders of magnitude smaller than that of the Earth and over 10,000 times larger than the upper limit of the lunar dipole. But what is the origin of Mercury's magnetism? The maximum field observed in the vicinity of the planet by Mariner 10 was 400 nT which, being 20 times greater than the interplanetary field, led Ness *et al.* (*Nature*, 255, 204; 1975) to rule out induction processes as a source. It seems more likely, therefore, that the field is of internal origin.

And of the various theoretically possible internal sources, Ness and his colleagues favoured a currently active dynamo in what would have to be a very large iron-nickel core. They tentatively rejected remanent magnetisation of parts of the planet on the grounds that it is "difficult to construct a plausible sequence of events leading to the model in which the Mercurian magnetic field is explained in [those] terms". Moreover, shortly before this, Runcorn (*Nature*, 253,

701; 1975) had shown that the external dipole moment of a shell magnetised in the field of an internal dipole which has since disappeared is apparently zero.

But according to Stephenson (*Earth planet. Sci. Lett.*, 28, 454; 1976), remanent magnetism as the source of Mercury's dipole moment cannot be rejected so easily. His new analysis indicates that an outer magnetised shell will give rise to a residual dipole moment if there is a difference in permeability between the shell and the planetary interior or between the shell and free space. Assuming the Mercurian surface rocks to be similar to those of the Moon, it may then be shown that a magnetic shell could quite easily account for the observed dipole moment without invoking ancient magnetising fields of more than a few gauss, an iron content of more than a few percent, or a shell thickness of more than a few tens of kilometres.

In short, contrary to previous belief, it is not necessary to conclude from the magnetic field observations that Mercury still has an active dynamo. The internal source options remain open.

widespread globally and are far from being a threatened species and yet which have received an inordinate amount of attention from British ornithologists because they have recently been able to invade, or reinvade, these islands as breeding birds. Would we not do better, Gooders argues, to concentrate on our ornithological resources which are truly of international importance, particularly in the conservation of such habitats as our estuaries which are vital for many species of wildfowl and wader.

One must sympathise with Gooders' view, and it is true that the British are not alone in this parochial attitude, nor is it confined to the ornithologists. In Estonia biologists pride themselves on having the mute swan (*Cygnus olor*) as a breeding species and avidly protect the single Soviet site where the cross-leaved heath (*Erica tetralix*) is found growing (the species is abundant in more oceanic areas of western Europe). The Norwegians take delight in the gorse effort in trying to rid himself of the same species. The British botanist (*Ulex europaeus*) which just maintains itself in the south-western parts of their country, whilst the British hill farmer spends much time and

fervently defends the few British plants of the military orchid (*Orchis militaris*) yet the plant is relatively frequent in parts of central Europe.

Indeed, the botanist is probably more regionally orientated than the ornithologist; the old county boundary is often his horizon. The Kentish man is proud of bog asphodel (*Narthecium ossifragum*) because it is rare in the south east of Britain, though common in the west, and in what was Warwickshire the crowberry (*Empetrum nigrum*) is prized, having only one station in the county, yet it is common further north.

But we can really expect more of mortal, irrational man? Can one really expect people to be concerned about species which may be threatened on a global scale and yet, for them, will never be more than a picture in a wildlife magazine? For the panda, or the tiger it may be possible, but for the bald ibis unlikely. The average subscriber to conservation bodies and fund-raising organisations cannot think in international terms because his main concerns are with the local situation. His reasons for desiring conservation are not coldly scientific but are aesthetic and emotive. □

articles

Radiocarbon chronology for early Maya occupation at Cuello, Belize

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Radiocarbon dates from excavations at the prehistoric Maya site of Cuello, Belize, indicate occupation around 2600 BC in calendar years, antedating the hitherto earliest known occupation of the Yucatan Peninsula by some 1,700 yr and demonstrating that sedentary pottery-using communities in the Maya lowlands may be as early as any in Mesoamerica.

WE report here a series of radiocarbon determinations which place the origins of Maya settlement and civilisation in the Yucatan Peninsula back in the third millennium BC, some 1,700 yr earlier than the first occupation known until now¹. Dating of the early phases of occupation of the Maya site of Cuello indicates a sedentary pottery-using settlement there by 2,000 yr b.c., in radiocarbon years, 2600 BC on the basis of the bristlecone pine calibration of radiocarbon chronology².

Background

Maya civilisation flourished in the Yucatan Peninsula and the adjacent highlands and Pacific slope through most of the first millennium AD, the Classic Period of greatest florescence being defined by the existence of dated inscribed monuments at about AD 250–900. Before this was a Preclassic or Formative Period going back to the earliest sedentary settlement of the Maya area and Mesoamerica generally, and formally defined as beginning in 2000 BC when it succeeded the long Archaic Period³.

During the Formative Period, settled village agriculture became the major means of subsistence in Mesoamerica, with the use of pottery beginning between 2,500 and 2,000 yr b.c., in highland and Pacific Mexico. On this base, complex societies arose towards the end of the second millennium BC, and the Olmec civilisation emerged in the Gulf Coast lowlands of Mexico after 1,200 yr b.c. The earliest evidence for settlement in the Yucatan Peninsula and Usumacinta basin to the east is based on a single radiocarbon date of 660 yr b.c., 850 BC on the bristlecone pine calibration (UCLA-1,437; 5382b 2,610 ± 75 yr b.p.) from the earliest Real Xe phase of the occupation of the major site of Seibal, Guatemala⁴, which had Olmec associations. The determinations listed below demonstrate the contemporaneity of occupation of the Maya lowlands with the Olmec to the west, and, in the priority of the earliest date, provide another possible source for Olmec culture.

Cuello

The Cuello site, located in northern Belize at approximately 18°05'N, 88°35'W, is one of a number of Maya ceremonial centres excavated over the period 1973–75 by the Corozal Project, a joint venture of the British Museum and Cambridge University. Excavations in 1975 (supervised by Pring) revealed a deep stratigraphic sequence in Operation 17B of plaster platforms and midden deposits with bedrock at ~4.70 m below ground level; the middens contained partially burnt wood which has been used for the bulk of the radiocarbon determinations reported here, although one date (Q-1,476) is directly associated with a burial. The determinations were carried out at the University of California (UCLA) by Berger and at Cambridge University by Switsur and Ward. The radiocarbon determinations were all carried out using gas proportional detectors filled with purified carbon dioxide produced by the oxidation of the specimens of partially burnt wood from the excavations. The samples were all rather small, and where they yielded insufficient gas for filling the counters, additional radioactively inert carbon dioxide was added. Each sample contained traces of plaster, and this was eliminated by treatment with dilute mineral acid. The calculated radiocarbon dates quoted are based on the 'Libby' half life for radiocarbon of 5,568 ± 30 yr.

All of the samples relate archaeologically to the Formative Period, before the institution of the practice of erecting dated stone monuments, and thus no equivalent dates in the Maya calendar are quoted, although for purposes of articulating the Christian and Maya calendars, we accept the Goodman–Martínez–Thompson correlation. Dates in calendar years BC are quoted here on the basis of the MASCA calibration and that recently advanced by Clark⁴, and together with the radiocarbon determination, excavation level and associated ceramic complex are displayed in Fig. 1. Determinations for the upper part of the stratigraphic sequence (levels 1–14) were made at Cambridge, those for the lower part (levels 15–28) at UCLA; the two series are mutually consistent.

The uppermost levels (1–2) of the excavation Operation 17B contained material of the Early and Late Classic Periods (AD 250–900), overlying a plaster floor (3) which covered a sequence of Formative Period deposits⁵. Levels 3–5 contained pure Late Formative ceramics of the Cocos Chicanel ceramic complex and levels 6–8 Cocos material mixed with ceramics of the

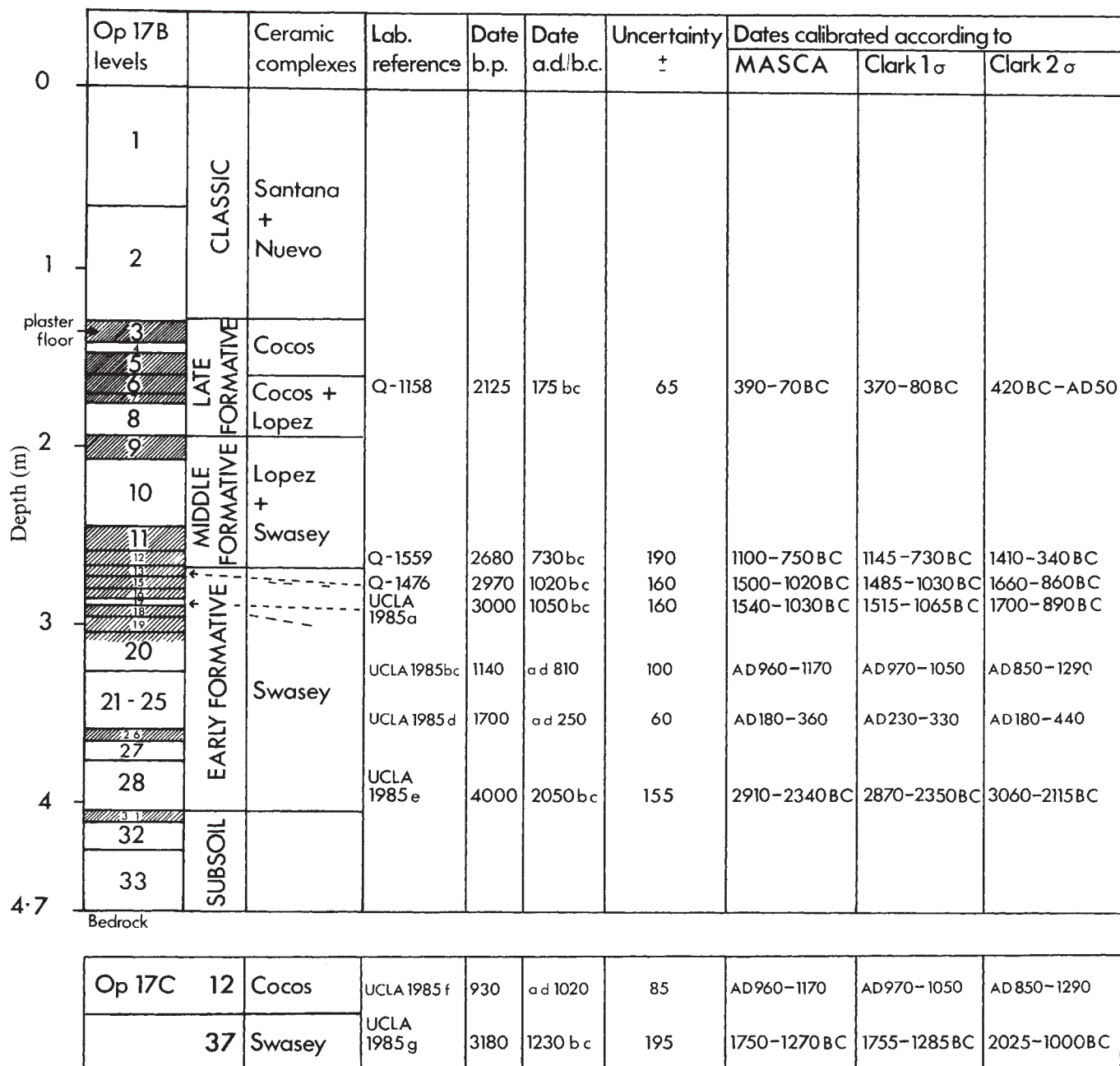


Fig. 1 Radiocarbon dates from Cuello, Belize. The dates are ranged against a scaled stratigraphic diagram (left) and major

period and ceramic complex designations. Dates considered archaeologically unacceptable are printed in smaller type.

preceding Lopez Mamom complex. A date for a sample of burnt wood from level 6 of $2,125 \pm 100$ yr b.p., 175 yr b.c. (Q-1558) falls well within the accepted span of 300 BC-AD 250 for the Late Formative Chicanel sphere, and the calibrated date is close to the radiocarbon result.

Levels 9-12 contained Middle Formative Lopez ceramics mixed with those of the Early Formative Swasey complex. The result of ^{14}C determination for a small sample of burnt wood (which had to be diluted with inert CO_2 for counting) from level 12 yielded a date of $2,680 \pm 200$ yr b.p., 730 yr b.c. (Q-1559), calibrating to ~ 910 bc. The formal lower limit of the Middle Formative has previously been interpreted as ~ 800 bc, but the Real Xe material from Seibal also has a calibrated radiocarbon date beyond this limit (of ~ 850 bc); consequently on the basis of the chronometric evidence we propose that the limit should now be taken as at least 1000 bc.

Apart from a pit (level 21) penetrating down through levels 13-26 and containing Lopez complex ceramics, and a burial (level 14) cut into the plaster platform level 13, all subsequent

stratigraphic levels in the excavation contained ceramics of only the Early Formative Swasey complex, the first indisputably Early Formative material to be found in the Maya Lowlands.

Level 14, a grave containing a single burial with three pottery vessels and a block of flint in place of a skull, yielded a sample of burnt wood from which a date of $2,970 \pm 160$ yr b.p., 1,020 yr b.c. (Q-1476) was obtained, calibrating to ~ 1275 bc. Levels 13, 15 and 16 were three superimposed plaster surfaces, below which, level 17, of occupation debris, yielded a further sample of burnt wood giving a date of $3,000 \pm 160$ yr b.p., 1,050 yr b.c. (UCLA-1985a), calibrating to 1320 bc; the Cambridge and UCLA dates for these two closely spaced levels are mutually consistent.

Levels 20 and 22 each yielded a very small burnt wood sample, which were amalgamated to give a date of $1,140 \pm 100$ yr b.p., a.d. 810 yr (UCLA-1985bc), calibrating to AD 820. This date lies in the Late Classic Period, and is not archaeologically acceptable in view of the association with Early Formative ceramics. The level immediately below, 23, yielded a burnt

wood sample which gave a date of $1,700 \pm 60$ yr b.p., a.d. 250 yr (UCLA-1985d), calibrating to AD 280. This date falls at the end of the Late Formative, and is again archaeologically unacceptable on the basis of the associated ceramics. Both UCLA-1985bc and UCLA-1985d are also out of sequence with the later dates Q-1558, Q-1559, Q-1476 and UCLA-1985a, and the earlier date UCLA-1985e, which are consistent with each other, the stratigraphic sequence and the associated ceramics. We must therefore set aside these dates, and suggest that later disturbance, natural or anthropogenic, brought xylic material into these early levels; excavation of a larger area of the site in 1976 may provide evidence of this postulated disturbance.

Level 28 was a primary midden containing large quantities of Swasey Complex pottery, mollusc shells, animal bones and carbon flecks. It was sealed by the unbroken superimposed plaster surfaces levels 26 and 27, and partly overlay a rough limestone surface (level 31), which together with the rest of the midden deposit rested on the old land surface, level 32. The midden was 25–30 cm thick over the whole area of the excavation and represents the debris from a substantial and undoubtedly sedentary occupation of the site. A sample of burnt wood gave a determination of $4,000 \pm 155$ yr b.p., 2,050 yr b.c. (UCLA-1985e), calibrating to ~ 2600 bc.

In another excavation at the site, Operation 17C, a major building recently half-destroyed by bulldozing for road metal was found to date to the terminal phase of the Late Formative, $\sim$ AD 200–250, on ceramic evidence. A construction level (12) yielded a sample of burnt wood which gave a determination of 930 ± 85 yr b.p., a.d. 1,030 yr (UCLA-1985f), calibrating to AD 1,050. This date is archaeologically unacceptable, and could have resulted from either root penetration when the site was abandoned after the Late Classic collapse of Maya civilisation⁶, or from an archaeologically undetected phase of Postclassic activity at the site.

Below the structure, in a level (37) of occupation material overlying limestone bedrock, a sample of burnt wood was recovered which gave a determination of $3,180 \pm 195$ yr b.p., 1,230 yr b.c. (UCLA-1985g), calibrating to 1520 bc. This gives an approximate date to a burial sealed by level 37 and lying in a pit cut into bedrock, which was accompanied by five pottery vessels⁶.

Discussion

The six acceptable determinations from the Cuello site together document a Formative sequence which, at its latter end, is compatible with what is already known about Maya Lowland and Mesoamerican chronology, but which, at its beginning, takes the prehistoric occupation of the Yucatan Peninsula back well into the third millennium bc. Although no other site in northern Belize has so far yielded similarly early radiocarbon dates, ceramics of the Swasey complex, which we must date to roughly 2,000–1,000 yr b.c. or 2600–1250 bc, have been recovered in Corozal Project excavations from three other sites, Nohmul, San Estevan and Santa Rita, mixed with later material but clear evidence of occupation at this early date. The existence of intensive agriculture utilising man-made raised fields along river and swamp margins a few km to the west by the end of the Swasey phase, is indicated by a radiocarbon date of $1,110 \pm 230$ yr b.c. (I-7877A) for a trimmed post associated with such fields, calibrating to ~ 1400 bc, and maize pollen from a core taken at Laguna de Cocos may date to ~ 1800 bc⁷. In addition a possible early lithic site has been reported at Richmond Hill (D. Puleston, unpublished) only 3–4 km from the Cuello site, and although it remains undated at present an association with the Swasey phase inhabitants of Cuello is possible.

Data from pollen cores taken from Laguna de Petenxil in El Peten, Guatemala, 150 km south-west of the Cuello site, have suggested an agricultural occupation of that region $\sim 2,000$ yr b.c. on the basis of radiocarbon determination Y-1285 of $3,990 \pm 160$, 2,040 yr b.c., calibrating to ~ 2590 bc⁸, and this, has been extrapolated on the basis of sedimentation rates to suggest

possible human interference with the vegetation of Peten $\sim 4,000$ yr b.c. (F. Wiseman, unpublished). This last proposal is still speculative; the Petenxil determination is, however, consistent with those from Cuello, and sedentary agricultural occupation of several areas of the rainforest zone by 2500 bc now seems likely. Such a date would accord with the still contentious glottochronological date for the splitting off of the Mayan language group.

On a broader Mesoamerican basis the radiocarbon chronology for Cuello may be compared with those for the Olmec area and the Central Mexican highlands. The latter part of the Swasey phase as defined by UCLA-1986g, UCLA-1985a and Q-1476 overlaps within 1σ the Bajío, Chicharras and San Lorenzo phases of the major early Olmec site of San Lorenzo Tenochtitlan⁹: the range for Cuello is 1,420–860 yr b.c., for San Lorenzo, 1,430–780 yr b.c. on 14 determinations. This range also overlaps the Tlatilco 'Olmec' and the Ayotla, Bomba and Justo phases in the Valley of Mexico, where the 1σ range of 5 determinations is 1,350–850 yr b.c. (ref. 10).

In the Maya highland zone the site of Chalchuapa in El Salvador has yielded two widely spaced dates for the Early Formative Tok complex of $2,790 \pm 60$ yr b.p., 840 yr b.c. (P-1551), calibrating to 1020 bc and $3,610 \pm 60$ yr b.p., 1,660 yr b.c. (P-1807), calibrating to ~ 2050 bc¹¹. The later of these overlaps the latter end of the Cuello triad at the 1σ level, whilst the earlier lies between and overlaps UCLA-1985e and UCLA-1985g on 2σ .

The earliest ceramics so far reported in Mesoamerica are from the Puerto Marquez site on the Guerrero coast¹² where a determination of $4,390 \pm 140$ yr b.p., 2,440 yr b.c. (Humble Oil Co; no laboratory number quoted), calibrating to ~ 3160 bc was obtained for a level containing mainly unslipped pottery termed 'Pox pottery'. Similar pottery was found in the Purron phase in the Tehuacan Valley in highland Mexico and placed by interpolation of dates at $\sim 2,300$ yr b.c. (2970 bc)¹³.

Earlier ceramics are known from north-western South America, from Colombia and Ecuador¹⁴, and from Panama at 4,090 yr b.p., $2,140 \pm 75$ yr b.c. (Y-585), 2750 bc¹⁵. This pottery includes sophisticated slipped wares, as does the Swasey complex, while the Pox and Purron ceramics are mainly unslipped; the sample of Swasey material currently available is too small for dogmatic pronouncements on its resemblance and possible relationship to either the South American or the Mexican pottery to be made. Since the dates for Pox, Purron and Swasey all overlap at the 2σ level, it is an open question as to whether the earliest ceramics in Mesoamerica come from the Pacific or Atlantic coasts, or from the intervening highlands, and whether they are autochthonous or of ultimately, and perhaps not distantly, South American inspiration.

We thank the Trustees of the British Museum, the Research Committee of the British Academy and the Leverhulme Trust Fund for funding the project, which was based at the Centre of Latin American Studies, Cambridge, and directed by N.H.

Received February 9; accepted February 25, 1976.

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Genetic hypertension in rats is accompanied by a defect in renal prostaglandin catabolism

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Noradrenaline releases prostaglandins in the kidney, and in rats these augment rather than reduce vasoconstriction produced by the amine. Homogenates of kidneys of New Zealand rats inbred for hypertension exhibit lower prostaglandin inactivation by 15-hydroxydehydrogenase than controls. At the same time, augmentation of noradrenaline vasoconstriction by the released prostaglandin is exaggerated. This biochemical defect could be the inherited abnormality primarily responsible for the development of hypertension in these animals.

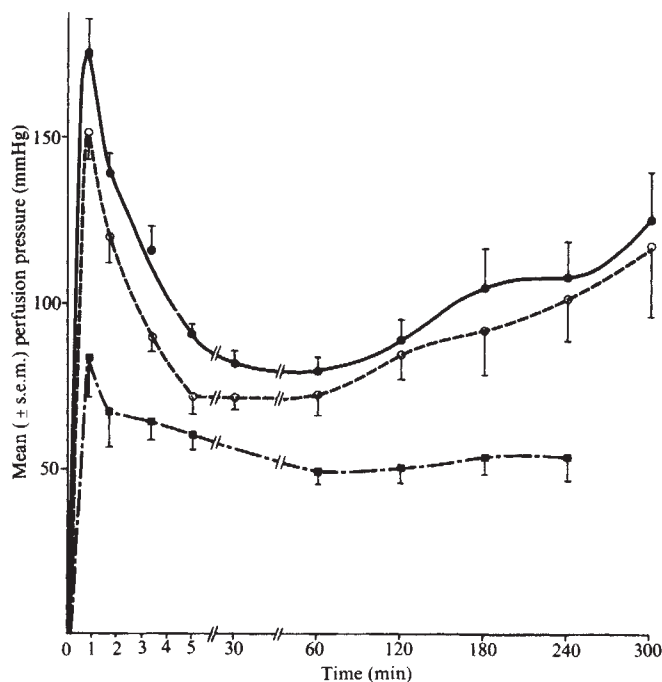
In 1958 Smirk and Hall¹ reported that hypertension can be inherited in rats by selectively inbreeding animals with above average blood pressures. The strain which they developed, the New Zealand, genetic hypertensive (GH) rat has been studied extensively and similarities looked for between this model of the disease and essential hypertension in man². The cause of hypertension is unknown in either species. Malik and McGiff³ found that, in contrast to other species, prostaglandins of the E series cause vasoconstriction in the kidneys of rats. They also augment adrenergic transmission and potentiate the vasoconstrictor effects of noradrenaline. These results suggest that renal prostaglandins could contribute to the development of hypertension in the rat, as an increase in renal vascular resistance might be an initiating factor in the pathogenesis of the disease⁴. They also question the use of hypertensive rats as a model of human essential hypertension. Prostaglandin E₂ (PGE₂) is the principal renal prostaglandin (PG)^{5,6} and is released from the kidney by pressor hormones^{7,8}. We have, therefore, studied interactions of noradrenaline and PGE₂ in the renal vascular bed of normotensive and GH rats. Metabolism of PGs by kidneys from the two types of rat was also investigated to determine whether abnormal PG synthesis or degradation could contribute to the increased renal vascular reactivity which occurs in GH rats⁹.

Male GH rats of the New Zealand strain of 17–19 weeks of age were used; their mean body weights ($\pm$ s.e.m.) were 242.1 ± 6.5 g and kidney weights were 1.56 ± 0.04 g per pair. We used as control rats a Charles River strain which, like GH rats, originated from the Wistar strain. As the GH rats weighed less than normotensive control rats of the same age, kidneys were used from controls of either similar age (12 weeks) or body weight to those of GH. Systolic blood pressures, measured indirectly from the tails of rats anaesthetised lightly with ether before removal of the kidneys, were 160–190 mm Hg in GH rats and 110–130 mm Hg in normotensive rats.

Animals were anaesthetised with chloroform and the lower alimentary tract was excised after tying and severing the superior mesenteric, coeliac, spermatic and suprarenal arteries. Following intravenous heparin (100 IU per 100 g) and ligation of the aorta just below the coeliac artery stump, the kidneys were flushed with 5 ml oxygenated Krebs' solution by way of a cannula inserted into the abdominal

aorta. The kidneys, attached to the cannula by the renal arteries and aorta, were rapidly transferred to the perfusion apparatus where they were hung from the aortic cannula in a covered water-jacketed glass container and perfused with oxygenated (95% O₂, 5% CO₂) Krebs' solution at 37 °C by a Watson-Marlow roller pump delivering 20 ml min⁻¹. This rate of perfusion flow, although greater than estimated by us to occur *in vivo*, has been found to prolong the useful life of similar preparations¹⁰. Furthermore, basal perfusion pressures were found to approximate more closely to normotensive blood pressures for rats *in vivo* (our unpublished observations). Perfusion pressure was measured by means of a Bell and Howell transducer placed proximal to the kidneys. The renal effluent was continuously assayed for PG-like activity by allowing it to superfuse in series a rat stomach strip, chick rectum and rat colon using the method of Ferreira and Vane¹¹. Specificity and sensitivity of the assay tissues for PGs were increased by adding a mixture of pharmacological antagonists¹² and indomethacin ($1\text{--}2 \mu\text{g ml}^{-1}$) into the renal effluent before it superfused the tissues. Changes in length of the assay tissues were detected using Harvard transducers (type 386) and transcribed by a

Fig. 1 Graphs of mean pressures developed in the system at intervals after starting the perfusion of hypertensive or normotensive rat kidneys *in vitro*. Normotensives of body weight similar to those of GH were chosen as controls as their perfusion pressures were also similar. —, Genetic hypertensive ($n = 19$); ----, Weight-matched normotensive ($n = 12$); - · - · -, Age-matched normotensive ($n = 6$). The number of preparations used in each group is given in parentheses.



Rikadenki KA-41 pen recorder. Drugs dissolved in saline were administered into a port situated proximal to the roller pump. Indomethacin was dissolved initially in 1 ml Tris buffer (pH 8) and the final concentration achieved by dilution with distilled water.

Selection of controls

Figure 1 compares changes, with time, of basal perfusion pressure of isolated kidneys of GH rats with those obtained from normotensive rats of either similar age or weight. The age-matched control group had a lower renal perfusion pressure, both at the start and at 5 h, than either the GH group or the weight-matched group. This difference could be accounted for by the differences in renal mass when identical rates of renal perfusion (20 ml min^{-1}) were used for the three groups. The kidneys obtained from GH and normotensive rats matched for body weight were comparable (1.6 g per pair), whereas those from age-matched normotensive rats weighed 2.3 g per pair, an increase in renal mass of about 40%. The heavier control kidneys had perfusion pressures which were 30–50% less than those of the other groups. Control rats were used with perfusion pressures similar to those of GH rats thus reducing the possibility that differences in vascular responsiveness between the two groups were a result of differences in basal tone of the vessels.

Release of PGs

Figure 2 is from an experiment in which different doses of noradrenaline were injected into the renal inflow. Dose-dependent increases in perfusion pressure were followed by the release of a substance which contracted the chick rectum and rat stomach strip superfused by the renal effluent. There was no effect on the rat colon. This pattern of activity could be matched by PGE_2 applied directly to the assay tissues. That PGs released from kidneys by noradrenaline were primarily of the E type, has been reported previously^{8,13}. When indomethacin ($1 \mu\text{g ml}^{-1}$) was infused into the kidneys, the rises in perfusion pressure induced by noradrenaline were substantially reduced and there was no release of PGE-like activity¹⁴.

Infusion of indomethacin into the kidneys of GH or control rats, although ensuring that its concentration in the superfusion fluid remained unaltered, was followed by relaxation of the assay tissues. This indicated that a resting output of PGE-like material had been abolished¹⁵. The level of basal release from the kidneys was estimated to be $0.1\text{--}1 \text{ ng ml}^{-1}$ as these concentrations of PGE_2 were required to restore the tone of the assay tissues.

When PGE_2 was infused into the indomethacin-treated kidney, in amounts ($100\text{--}300 \text{ pg ml}^{-1}$) which by themselves did not affect basal perfusion pressure, the pressor effects of noradrenaline were increased (Fig. 3a). This enhancement was sufficient to restore the effects of noradrenaline to pre-indomethacin levels. The potentiating effect of PGE_2 developed within 16–20 min and usually waned over a similar period after stopping the infusion. The results of these experiments are summarised in Fig. 3b, c where changes in renal perfusion pressure produced by noradrenaline in GH and normotensive rats are shown before and after inhibition of PG synthesis by indomethacin¹⁴, the latter in the presence and absence of an infusion of PGE_2 .

Indomethacin usually did not affect basal renal perfusion pressure in normotensive rats, whereas in GH rats addition of indomethacin to the perfusing medium resulted in small reductions of 5–8 mmHg in perfusion pressure in 60% of these experiments. The failure of indomethacin to produce greater effects on renal perfusion pressure is consistent with the relatively small basal PG efflux from the rat kidney. Such small amounts would have marginal effects on basal tone but might still potentiate the renal vasoconstrictor activity of noradrenaline.

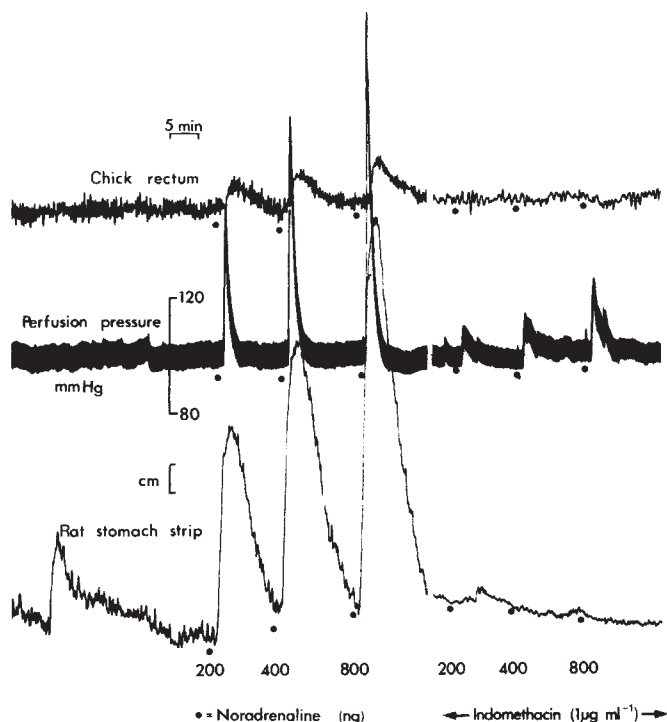


Fig. 2 The kidneys of a normotensive rat were perfused with Krebs' solution *in vitro* and the effluent allowed to superfuse assay tissues as described in the text. Indomethacin added to the perfusion fluid abolished the release of PGE-like activity, denoted by contractions of the chick rectum (top trace) and rat stomach strip (bottom trace), which normally accompanies the pressor responses (middle trace) evoked by noradrenaline. Moreover the rises in perfusion pressure produced by the amine were also markedly reduced in the presence of indomethacin.

In all these experiments we used the superfusion bioassay method to detect release of PGs by noradrenaline, to ascertain the effectiveness of indomethacin treatment in preventing release, and to determine the smallest dose of indomethacin which would achieve this effect. Since indomethacin inhibits PG dehydrogenase¹⁶ and phosphodiesterase¹⁷ in concentrations higher than those which affect synthetase, effects which by themselves could influence vascular reactivity, it was essential to select the minimal effective dose of indomethacin which inhibited prostaglandin synthetase. Our unpublished observations indicate that the concentration of indomethacin used ($1\text{--}2 \mu\text{g ml}^{-1}$) in this study does not substantially inhibit PG 15-hydroxydehydrogenase. These findings confirm those of Pace-Asciak¹⁶.

Comparison of the vasoconstrictor responses to noradrenaline administered by injection in random sequence revealed that the renal vasculature of GH rats, compared with that of normotensive rats, was about 50% more sensitive to noradrenaline and was more susceptible to indomethacin-induced attenuation of the constrictor action of noradrenaline (Fig. 3b, c). In normotensive rats, PGE_2 ($100\text{--}300 \text{ pg ml}^{-1}$) restored to pre-indomethacin levels the depressed renal vasoconstriction to doses of noradrenaline of 200 ng or greater, but not to low doses (50–100 ng). In contrast, similar concentrations of PGE_2 restored renal vascular reactivity of GH rats to low doses of noradrenaline, whereas restoration of responses to pre-indomethacin levels was incomplete to doses of the amine greater than 200 ng. The latter was related in part to the greater reduction in renal vascular responsiveness to noradrenaline produced by indomethacin in GH rats. Thus, the magnitude of PGE_2 -induced potentiation of doses of noradrenaline greater than 100 ng in the presence of indomethacin was similar in both types of kidneys. The failure of exogenous PGE_2 to restore

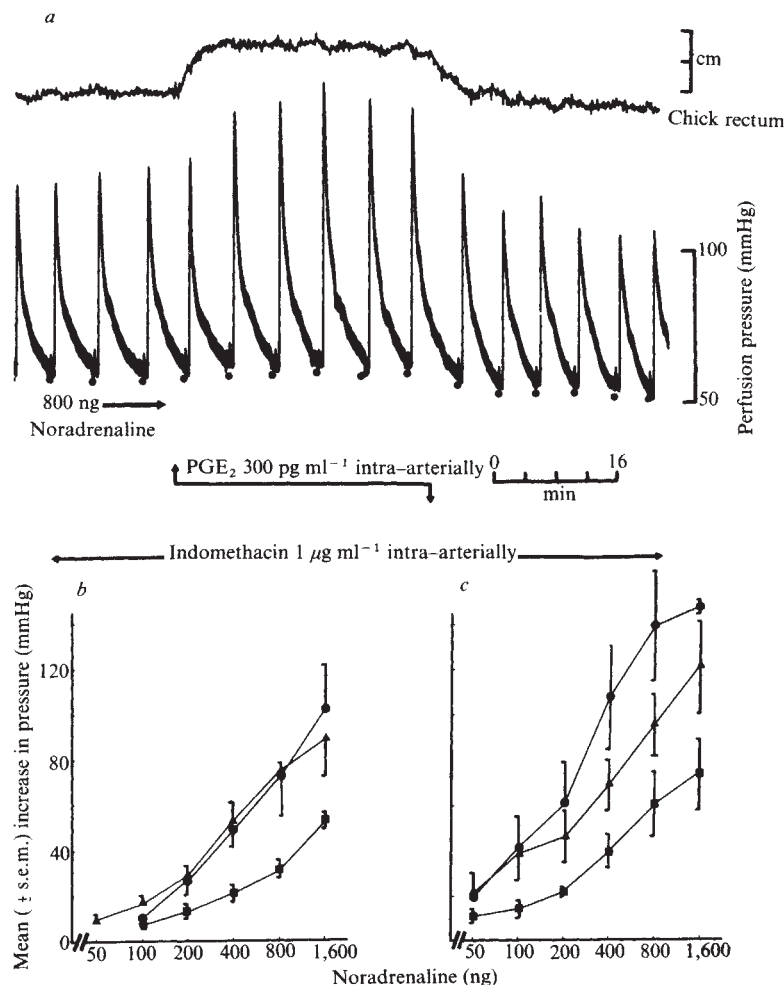


Fig. 3 *a*, Changes in perfusion pressure in response to intra-arterial noradrenaline (800 ng) injected at the dots, into the kidneys of a normotensive rat perfused *in vitro* with Krebs' solution containing indomethacin (for method see text). PGE₂, in amounts subthreshold for vasoconstriction, potentiated the vasoconstrictor responses to noradrenaline. The potentiation declined after withdrawal of the PG. *b*, *c*, Dose-response curves relating the vasoconstrictor responses to various doses of noradrenaline injected into the kidneys of normotensive (*b*, *n* = 7–10) or hypertensive (*c*, *n* = 7) rats *in vitro*. Addition of indomethacin to the perfusion fluid reduced in either group responses to each dose of the amine used. Thereafter infusion of PGE₂, in amounts subthreshold for vasoconstriction, augmented responses in either group. ●, Initial; ▲, indomethacin + PGE₂ (100–300 pg ml⁻¹); ■, indomethacin (1–2 µg ml⁻¹).

vasoconstrictor responses to noradrenaline may perhaps be a consequence of a reduced protein synthesis *in vitro* which would lead to lowered dehydrogenase concentrations¹⁸ and thereby less potentiation than one would otherwise expect.

Of the other PGs identified in the rat kidney⁵, PGF_{2α} in concentrations up to 10 ng ml⁻¹ did not affect the renal vascular responsiveness to noradrenaline, whereas PGD₂ resembled PGE₂ in its ability to potentiate the amine.

The finding that kidneys of normotensive rats were less sensitive to inhibition by indomethacin of the vasoconstrictor action of noradrenaline could indicate that a smaller contribution was made by released PGs to the vascular effects of the amine than was the case in GH kidneys. Indomethacin caused a larger absolute reduction in vascular reactivity to noradrenaline in kidneys of GH rats than in those of normotensive rats, the renal vasoconstrictor responsiveness to noradrenaline was least in normotensive rats after indomethacin treatment. The difference which was revealed by indomethacin between the kidneys of GH and normotensive rats in their vascular responses to noradrenaline, that is, when the contribution of PGs was eliminated, is important since it indicates that other factors, such as structural changes in the vascular wall¹⁹, also contribute to the increased vascular sensitivity to noradrenaline in GH rats.

Renal PG metabolism

Our results suggest that increased renal vascular reactivity to pressor hormones in the GH rat²⁰, could result partly from elevated levels of PGs. Indeed, the renal vascular responsiveness to noradrenaline was reduced by indomethacin to a greater extent in the GH rat than in the

normotensive rat (Fig. 3*b, c*). If increased levels of PGs are present, this could result from either increased synthesis or decreased degradation. Production of PGs by homogenates of kidneys of GH rats was, however, not different from normal as indicated by measurements of conversion of labelled arachidonic acid to PGs (Table 1). Moreover, no change could be detected in the pattern of production of PGs; that is, the rate of synthesis of PGE₂ was similar to that of PGF_{2α} in normotensive and GH rats; (PGE-PGF) ratios of 1.3 : 1 were obtained. As the activity of PGE₂ as a renal vasoconstrictor agent is considerably greater than that of PGF_{2α} (ref. 3), changes in patterns of synthesis of PGs without changes in their rate could influence vascular reactivity.

In contrast to the above findings, important differences were found between the kidneys of GH and normotensive rats in their abilities to degrade PGs to their 15-keto metabolites, the first and most important step in the metabolism of renal PGs²¹. This assay depends on the oxidation of ³H-PGE₂ by an enzyme, PG 15-hydroxydehydrogenase (PGDH), the activity of which is associated with a high speed supernatant fraction obtained from renal homogenates. Blackwell, Flower and Vane¹⁸ reported that the oxidation of ³H-PGE₂ by the enzyme obtained from this fraction was linear for the first 5 min during which time approximately one-half of the labelled substrate was metabolised. We have confirmed these findings. A marked reduction of PGDH activity in the kidneys of GH rats was demonstrated when compared to those of normotensive rats (Fig. 4). These differences in PGDH activity were evident when kidneys of normotensive rats of either similar weight or age to those of GH rats were used. Since an age-dependent difference in PGDH activity has been described

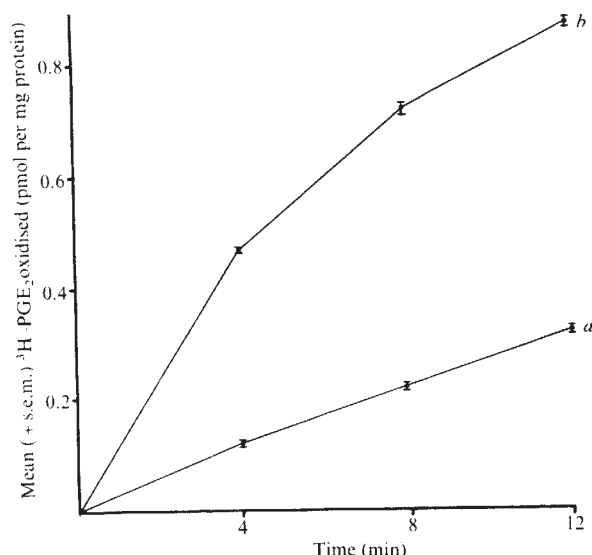


Fig. 4 Comparison of the activity of PGDH from the kidneys of GH (a) and normotensive (b) rats of similar weight ($n = 3$ for each point). Kidneys of male rats were homogenised in ice-cold 100 mM Tris buffer, pH 7.5. After centrifugation at 1,000*g* to remove cellular debris, the supernatant was recentrifuged at 100,000*g* for 1 h in a Beckman Spinco ultracentrifuge. Of the resultant supernatant, 1 ml containing the PGDH was added to a reaction mixture containing 280 pmol NAD⁺, 0.1 μ Ci ³H-PGE₂ and 28 pmol unlabelled PGE₂. After incubation at 37 °C for 4, 8 and 12 min the reaction was stopped by acidification to pH 3 with 1 N HCl. A control mixture containing boiled supernatant was similarly treated. Unreacted PGE₂ and its metabolites were extracted by mixing with 1.5 ml ethyl acetate. The phases were separated by centrifugation and 1 ml of the organic layer was removed and evaporated in a vacuum desiccator. The dried residue was then reconstituted in 30 μ l of chloroform; methanol (2:1) with unlabelled PGE₂ and 15-keto PGE₂ as markers. This was then spotted quantitatively on plastic-backed silica-gel plates and developed in an ethyl acetate-water-isooctane-acetic acid (11:10:5:2) system. The zones corresponding to the authentic prostaglandin markers were indicated by brief exposure to iodine. These zones were then cut out and placed in a scintillation vial containing 10 ml Diatol. The radioactivity in each zone was estimated by liquid scintillation counting on a Beckman LS-150 scintillation counter. c.p.m. were converted to d.p.m. using a quench correction curve and external standards. Results are expressed as the mean oxidation of PGE₂ to 15-keto PGE₂ per mg protein ($\pm$ s.e.m.) with time. Protein content of the supernatant fraction was determined by the Biuret method.

by Pace-Asciak²² it was important to use as controls age-matched kidneys to characterise changes in renal vascular reactivity. A decreased catabolism of PGs could lead to increased levels within the kidneys of GH rats, and thereby partially explain the enhanced renal vascular responsiveness to noradrenaline and the greater lowering of renal vascular sensitivity to noradrenaline produced by inhibition of PG synthesis in this species (Fig. 3b, c) PGE₂ released by noradrenaline potentiates the vascular action of the amine as well as having an additive vasoconstrictor action of its own. The latter is shown when large amounts of PGE₂ are infused in the kidney³. We obtained evidence supporting the uniqueness of the deficiency of PG metabolism in the GH rat by showing that another form of hereditary hypertension in the rat²³, the Japanese spontaneously hypertensive (SH) rat, was not associated with decreased renal PGDH activity (Table 1). Further, hypertension produced by constriction of one renal artery and of comparable severity to that in the GH rat, did not result in a similar derangement of PG metabolism in either the constricted or the untouched contralateral kidney. In three experiments oxidation of PGE₂ by PGDH was measured in kidney homogenates of renal hypertensive and normotensive rats. Hypertension (approximately 170 mmHg), was produced 5–7 weeks after constriction of the right renal artery using a silver clip

Table 1 Prostaglandin metabolism in normotensive and hypertensive rats

	Catabolism			Synthesis pmol PG per min per g kidney
	fmol PGE ₂ per min per mg protein			
	GH	GH	SH	GH
Hypertensive	76.2±2.9	46.0±6.0	397.3±5.7	17.29±1.26
	*201.2±3.3	*222.3±7.1	‡378.6±1.6	
Normotensive		†118.3±7.1	305.3±17.9	17.20±1.36

Kidneys of male rats were homogenised in ice-cold 100 mM Tris buffer, pH 7.5. To the homogenates were added 20 μ g each of PGE₂ and PGF_{2 α} to saturate the metabolising enzymes. Prostaglandin synthesis was determined using a radiochemical assay. 1 ml of the homogenate was added to the reaction mixture containing 0.1 μ Ci ¹⁴C-arachidonic acid 33 pmol unlabelled substrate, 10 μ l reduced-glutathione, 5 mM, and 10 μ l (–)-adrenaline, 5 mM. The reaction mixture was then incubated at 37 °C for varying times between 2 and 10 min, and the reaction was stopped by boiling for 2 min. A sample with a boiled enzyme preparation was used as control. The mixture was then acidified and the unreacted arachidonic acid and its products extracted and chromatographed on a silica gel TLC plate (Uniplat) with unlabelled PGE₂ and PGF_{2 α} as markers. The zones corresponding to the prostaglandins were scraped into a scintillation vial and the radioactivity determined (as for Fig. 5). The units of enzyme activity, fmol per min per mg protein or pmol per min per g kidney, were calculated from the difference in d.p.m. of each product relative to that of the control, the specific activity of the ¹⁴C-arachidonic acid or the ³H-PGE₂, the percentage extraction of each product, the aliquot ethyl acetate taken, the incubation time and the protein concentration or weight of the kidney.

$n = 3-5$

*Weight-matched normotensives.

†Age-matched normotensives.

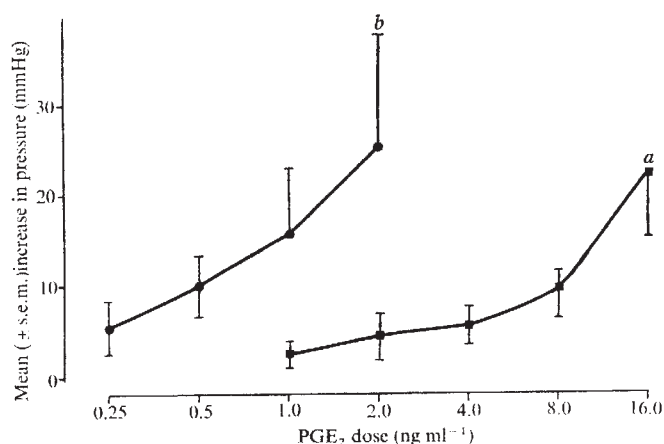
‡Japanese normotensives.

GH, New Zealand genetic hypertensive; SH, Japanese spontaneously hypertensive.

(diameter 0.23 mm) leaving the contralateral kidney untouched. The two kidneys were assayed for PGDH activity separately. Rats, similarly treated, which had not developed hypertension were used as controls. Differences in enzyme activity observed between the two types of kidney were small relative to those of GH and normotensives. The finding that normal renal PGDH activity occurs in rats with other types of hypertension suggests that the fault occurring in GH kidneys is not secondary to the high blood pressure.

If lower than normal degradation of PGs contributes to enhanced renal vascular reactivity in the GH rat, then

Fig. 5 Mean changes in perfusion pressure produced by PGE₂ infused for 8 min into kidneys of normotensive (a, $n = 9$) or hypertensive (b, $n = 9$) rats *in vitro*. Curves of hypertensives were found to be to the left of normotensives denoting greater vascular sensitivity to the PG.



it should be possible to show greater than normal vascular reactivity to exogenous PGE_2 in kidneys from these animals. We, therefore, infused PGE_2 in different concentrations and showed increased vascular sensitivity to PGE_2 in the kidneys of GH rats, as indicated by a lowering of the dose required to produce a threshold response (Fig. 5). There was also an approximately tenfold increase in responsiveness to its vasoconstrictor action when compared with its effects on the renal vascular bed of normotensive rats.

Prohypertensive activity

Rats with hereditary forms of hypertension have so far been considered as good animal paradigms of human essential hypertension¹⁹. Abnormalities of a system involved in blood pressure regulation have long been sought in genetically hypertensive rats². The vasoconstrictor effects of endogenously released PGs coupled with a relative lack of the inactivating enzyme, offer a biochemical basis for hypertension in the GH rat. At the same time, the fact that PGE_2 is vasodilator in the kidneys of most other species must call into question the use of hypertensive rats as a model of human disease.

Prostaglandins as modulators

As modulators, prostaglandins may amplify or attenuate the vascular actions of other hormones or neurotransmitters in concentrations which do not affect the vasculature directly. Thus, prostaglandins have been shown to amplify the renal vascular action of kinins²⁴ and attenuate those of angiotensin II and noradrenaline^{7,13,15}. There is an additional and less well known corollary of possible interactions between modulators and hormones, and one which has bearing on the concept that hypertension may develop in the face of normal activity of either the adrenergic nervous system or the renin-angiotensin system²⁵. That is, that an abnormality of the modulator, by itself, could result in elevated blood pressure and we suggest this is the case in the GH rat. Such an explanation accounts for the demonstration that, although the adrenergic nervous system is essential for maintaining elevated blood pressure in GH rats, there is no direct evidence for its activity being increased in this

species. Thus, the primary or initiating event would be increased levels of PGE_2 , or a similar product of PG synthetase, which results from a deficiency of the major catabolising enzyme in the face of normal rates of prostaglandin synthesis. As emphasised above, it is likely that this disturbance need only occur in the kidney for the development of hypertension. Recent studies on other visceral vascular beds of the rat, demonstrate, however, that one or more products of prostaglandin synthesis^{26,27} contributes to the vasoconstrictor actions of adrenergic stimuli. The present study is the second which indicates a genetically determined defect in prostaglandin metabolism, the first being a deficiency in synthesis demonstrated in the platelets of a patient with an haemostatic defect²⁸.

Received November 27, 1975; accepted February 26, 1976.

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Early Mesozoic rifting and fragmentation of the Cordilleran orogen in the western USA

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New data make possible the precise definition of the truncated edge of the southern part of the Cordilleran orogen in California, and lead to the identification of possible 'missing pieces' removed during the early Triassic. A probable reconstruction of the Palaeozoic Cordilleran orogen involving large scale lateral transport of major tectonic units is suggested.

MANY lines of evidence indicate that only part of the Palaeozoic Cordilleran orogen is preserved intact in the western USA. An inferred south-western segment of unknown dimensions that presumably extended south-west of California has been lost. North-west-trending Mesozoic arc-trench complexes developed adjacent to the raw edge of a

truncated Palaeozoic orogen^{1–5}. Few people have attempted to reconstruct the ancient orogen or to identify its dispersed fragments, although studies in the Canadian and Alaskan Cordillera have begun to suggest that certain Palaeozoic terrains may have originated far to the south^{6,7}, perhaps even adjacent to the truncated margin of the US Cordillera⁸.

Before missing parts of the Palaeozoic orogen can be identified confidently and restored to their original positions, it is necessary to bring into closer focus the truncated margin itself. Where is the edge, and what is the nature of the truncated terrains? If these questions can be resolved, it should be possible to specify the nature of the missing parts and, if they can be identified elsewhere, to outline a broad kinematic history of breakup of the orogen.

This article is composed of three parts. In the first part, I discuss current views of the relations of Palaeozoic ter-

rains of northern California and Oregon to their counterparts in Nevada. New data from the Sierra Nevada region of California and their bearing on the location of the truncated edge of the orogen are then presented. In the second part, I present arguments concerning the identity of displaced terrains. I conclude, in the third part, with a possible tectonic reconstruction and model for the truncation and fragmentation of the orogen during early Mesozoic time.

Accepted model of late Palaeozoic orogen

Several reviews envision island arc complexes in the northern Sierra Nevada and eastern Klamath Mountains of California, together with presumably equivalent units in eastern Oregon, as having fringed the Palaeozoic North American continent much as Cainozoic island arcs in the western Pacific festoon the south-eastern margin of Asia. The volcanic archipelago was flanked to the south-east by contemporaneous deep-water chert-argillite facies with minor limestone and mafic volcanic rocks that apparently accumulated in a marginal basin which separated the arc complex from the Cordilleran miogeocline. These marginal basin rocks were thrust south-easterly over shallow-water shelf facies (Fig. 1) during periods of contraction of the marginal basin^{4,5,8-10}.

This model is evidently appropriate for two distinct parts of the Palaeozoic. (1) Beginning in the Devonian, an arc developed on ultramafic basement in the eastern Klamath Mountains^{4,5,10} (1, Fig. 1) and on metasedimentary basement in the northern Sierra Nevada (2, Fig. 1), adjacent to what had formerly been a stable continental margin¹⁰. The presence of bluechist in the heart of the structurally complex north-westernmost part of the eastern Klamath Mountains¹¹ (3, Fig. 1), where rocks of Ordovician, Silurian and Devonian ages occur, suggests that the north-east-trending Palaeozoic arc complex (1, Fig. 1) was paralleled by a north-east-trending subduction complex, and therefore faced north-west.

Most workers have concluded that the arc terrain was flanked by a marginal basin to the south-east, but it is important to note that the marginal basin did not originate by back-arc spreading^{5,8,9}. During the late Permian-early related arc magmatism, well removed from the continental edge. The sedimentary fill of this trapped marginal basin was uplifted and thrust south-eastward on to the miogeoclinal prism as the marginal basin was closed during the late Devonian-early Mississippian Antler orogeny^{4,5}.

(2) During Permo-Carboniferous time, an analogous arc-marginal basin system developed in nearly the same position as the earlier couplet, but this time the marginal basin grew by back-arc spreading^{5,8,9}. During the late Permian-early Triassic Sonoma orogeny the sedimentary contents of the marginal basin were again uplifted and thrust on to the Antler orogenic belt^{4,5,8,9}.

Effects of the Antler orogeny have been obscured in places by the younger event, and evidence of a Devonian arc complex apparently exists only in northern California. The Permo-Carboniferous record is more complete, making possible more confident recognition of the various tectonic elements in Nevada, California and Oregon. In neither postulated phase of closure of the marginal basin is the fate of the presumed underlying oceanic crust known^{4,5,8,9}, but one hypothesis suggests that closure was accomplished by contemporaneous subduction of oceanic crust and obduction of sedimentary fill^{5,12}.

Sierran Palaeozoic rocks and their relation to the orogen

The foregoing model of arc-marginal basin tectonics is based almost entirely on data from north-central Nevada and northern California. A large terrain of Palaeozoic metasedimentary rocks occurs in part between these better known areas, in the western Sierra Nevada (Fig. 1), and is

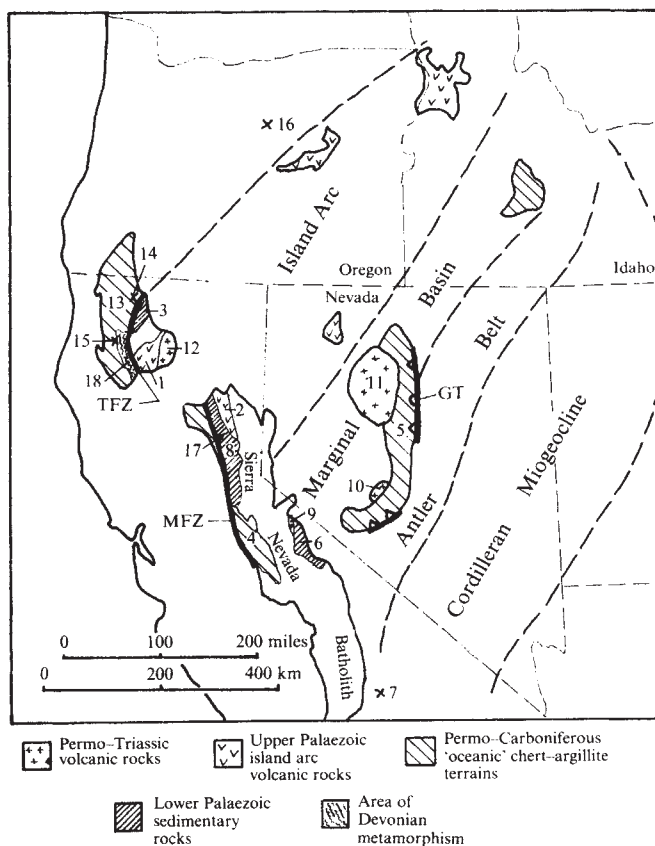


Fig. 1 Sketch map showing inferred tectonic elements of the Cordilleran orogen and specific localities, 1-18, discussed in the text; relations in eastern Oregon are uncertain in detail and highly schematic as shown. The map is modified, in part, from Fig. 1 of ref. 8 and Fig. 4 of ref. 4. GT, Golconda thrust; MFZ, Melones fault zone; TFZ, Trinity fault zone.

separated from pre-Mesozoic rocks of Nevada by the Mesozoic Sierra Nevada batholith. Although little is known of specific stratigraphic and structural relationships between Palaeozoic rocks on opposite sides of the Mesozoic batholith, there is no evidence that the batholith occupies a major tectonic boundary.

The Sierran Palaeozoic terrain lies at a high angle to the north-eastern tectonic trends of the Palaeozoic Cordillera, and its southern half (4, Fig. 1) occupies a "back-arc" position analogous to that of probable marginal basin rocks of north-central Nevada (5, Fig. 1; discussed below). The part of the Sierran terrain north of about latitude 39° is here considered older sedimentary basement of the above-mentioned island arc complex. Recent field studies¹³⁻¹⁵, discussed below, have revealed major lithological and structural contrasts between the southern part of the Sierran belt (known as the Calaveras Formation) and the northern part of the belt (to which the name Shoo Fly Formation is applied).

The Calaveras Formation (4, Fig. 1) is a structurally complex assemblage of argillite, chert, subordinate quartzite and greenstone, with several large, lenticular masses of strongly recrystallised limestone. Recent work (refs 13, 14 and my unpublished data) indicates that the Calaveras Formation consists of two major units. The lower unit is a very thick, extensive mass of chaotic submarine slide deposits consisting primarily of fragments and blocks of chert in an argillaceous matrix. Most exposures can be termed diamictite, and bedding is only rarely preserved. The chaotic rocks were deposited on a structurally coherent sequence of mafic pillow lava, pyroclastic rocks, and interbedded shale. These rocks are overlain on the east by an upper sequence of quartzites (ref. 16 and my unpublished

data) in which graded beds indicate tops to the east, and interbedded olistostromes. All rocks of the Calaveras Formation have been penetratively deformed, producing an east- to north-east-plunging extension lineation and a regional schistosity. The Calaveras Formation may be Permo-Carboniferous, but faunal evidence is meagre and does not preclude the existence of rocks of other ages. The penetrative deformation predates the development of Jurassic structures and the intrusion of Jurassic plutons, and may be late Palaeozoic or early Mesozoic^{13,14}.

As Speed suggested¹⁷, the Calaveras Formation is probably correlative with the Havallah sequence in north-central Nevada (5, Fig. 1), which has been interpreted by other workers to have accumulated in the late Palaeozoic marginal basin^{4,5,8,9}. My reconnaissance of parts of the Havallah sequence has revealed the existence of widespread submarine slide deposits consisting primarily of chert-argillite diamictite with assorted blocks of chert, sandstone, limestone and greenstone. In addition, evidence of penetrative deformation is present. The lower chert-argillite-greenstone units of the Havallah sequence are overlain by an upper quartzitic sequence^{18,19}. These highly distinctive lithological and structural characteristics are identical to those of rocks of the Calaveras Formation. The Havallah sequence contains fossils of middle Pennsylvanian to early Permian age⁸. During the Sonoma orogeny, these marginal basin sedimentary and volcanic rocks were thrust south-eastward along the Golconda thrust on to rocks of the Antler orogenic belt^{4,5,8,9,17}.

Owing to their analogous palaeogeographic setting, remarkably similar lithology and structure, and probably similar age¹⁷, I suggest that the chaotic chert-argillite facies and quartzites of the Calaveras formation are part of the marginal basin assemblage represented by the Havallah sequence. The chaotic nature of parts of both sequences probably resulted from repeated slumping in this tectonically unstable environment; shallow-water limestones in both areas were introduced into deep-water environments by large scale slumping. The penetrative deformation of both terrains probably represents shortening during the Sonoma orogeny when the marginal basin was partially closed. Rocks representative of the late Palaeozoic marginal basin can thus be considered to extend as far west as the Melones fault zone in the western Sierra Nevada (Fig. 1).

Like the Havallah sequence, rocks of the Calaveras Formation may be largely allochthonous, and if so they are separated from miogeoclinal Palaeozoic rocks of the eastern and southern Sierra Nevada (6, Fig. 1) by the projected Golconda thrust, whose actual trace has been obliterated by the Sierra Nevada batholith. The Golconda allochthon may in addition include part of the Garlock Formation (7, Fig. 1) as suggested by Burchfiel and Davis^{4,5}, requiring projection of the thrust 300 km south of its known extent in north-central Nevada.

Metasedimentary rocks in the northern half of the Sierran belt (8, Fig. 1) are known as the Shoo Fly Formation^{20,21}. These rocks are considerably older than the Calaveras Formation and may be Silurian or older^{15,16,21}. They are unconformably overlain by Upper Devonian volcanic rocks^{22,23} (2, Fig. 1) which form the basal part of the volcanic arc discussed above^{2,4,5,8-10}. The Shoo Fly is made up of slate, phyllite, graywacke and distinctive quartzose sandstone, bedded chert and rare thin limestone beds^{15,21,23}. Its thickness is unknown as it is tightly folded and penetratively cleaved.

The Shoo Fly comprises a thick, deformed metasedimentary pile on which the Devonian-Permian arc complex developed^{2,4,5,8-10}, and thus contrasts with the ultramafic basement of the arc in the Klamath Mountains. It resembles lithologically the siliceous facies¹⁰ of the early Palaeozoic eugeocline to the south-east. The entire northern Sierra Nevada terrain and that of the eastern Klamath Mountains,

including both younger arc rocks and older metasedimentary and ultramafic basement, may have been rifted north-westerly away from the eugeocline during growth of the late Palaeozoic marginal basin to the south-east.

Widespread Permo-Triassic volcanism

Thick sequences of silicic volcanic rocks or probable Permo-Triassic age occur in many parts of the Cordilleran orogen, within both the former marginal basin and the arc complex proper.

In the eastern Sierra Nevada, the late Palaeozoic Koip sequence²⁴⁻²⁶ (9, Fig. 1) consists of andesitic and rhyolitic lava and pyroclastic rocks²⁶. Age assignment is based on two Rb-Sr whole-rock isochron ages: 240 Myr (ref. 24) and 250 Myr (ref. 25). These rocks, together with similar rocks of the Permo-Triassic(?) Excelsior Formation²⁶ within the Sonoma orogenic belt^{19,27} (10, Fig. 1), may occupy a structural position analogous to that of the Permo-Triassic Koipato sequence of north-central Nevada (11, Fig. 1), which consists of more than 3 km of rhyolitic and andesitic lava and pyroclastic rocks^{8,19}. The Koipato has yielded fission-track and lead-alpha ages that average about 250 ± 40 Myr (ref. 28). Koipato rocks were erupted and deposited on the Havallah sequence, evidently after thrusting^{8,17,19}. Although precise age equivalence cannot be demonstrated, the Koip, Excelsior and Koipato collectively

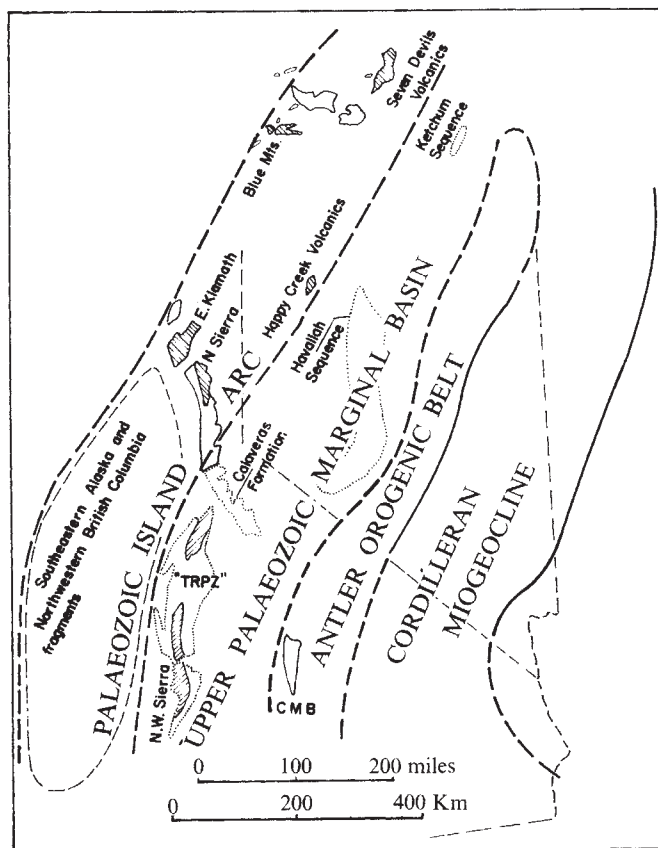


Fig. 2 Proposed tectonic reassembly of southern part of Cordilleran orogen for Permian time, before the Sonoma orogeny, which involved shortening and thrusting of marginal basin fill on to the Antler orogenic belt. Terrains north-east of the line of truncation have been shifted 50 km north-west of their present locations to eliminate effects of the Sonoma orogeny; Mesozoic and Cainozoic displacements have not been treated. Arc rocks of the eastern Klamath Mountains have been shifted 60 km closer to those of the northern Sierra Nevada⁵³. The dot pattern outlines chert-argillite terrains which were once part of the marginal basin. North-east ruling marks probable remnant arcs within the marginal basin; north-west ruling marks arc volcanic sequences in the autochthonous part of the Palaeozoic island arc. TRPZ, western Palaeozoic and Triassic belt; CMB, central metamorphic belt. See text for discussion.

indicate extensive Permo-Triassic silicic volcanism within the Sonoma orogenic belt.

Notable Permo-Triassic volcanic rocks much like the above units are found, in addition, in the eastern Klamath Mountains, overlying Devonian to Permian arclike volcanic rocks discussed earlier (12, Fig. 1). These units, the Dekkas Andesite and Bully Hill Rhyolite, constitute up to 2 km of lava and pyroclastic rocks²⁹, and perhaps should be regarded simply as continued arc volcanism. Nevertheless, the widespread Permo-Triassic andesitic and rhyolitic volcanism within both former arc and marginal basin settings may be indicative of a transition to extensional tectonics following the Sonoma orogeny¹⁷, preliminary to rifting within the orogen. Burchfiel and Davis^{5,12}, however, consider such volcanism to be arc volcanism which resulted from subduction of the oceanic crust on which the Havallah sequence was presumably deposited⁸.

Truncated edge in central California

As shown here, recognisable elements of the late Palaeozoic Cordilleran marginal basin continue south-westerly across the Sierra Nevada batholith into the western Sierra Nevada. Such rocks do not extend beyond the Melones fault zone, the major tectonic boundary of the region, and which apparently represents a collision boundary between the rifted Palaeozoic margin and a Jurassic island arc¹⁵. The Melones fault zone has been interpreted as a high-angle reverse fault³⁰, strike-slip fault³¹, and as a thrust fault³². Limited exposures of melanges with Permian limestones occur west of the Melones fault zone³³, but their Tethyan faunas³⁴, and tectonic setting within a Jurassic island arc¹⁵, suggest that these rocks did not originate within the Cordilleran orogen. The Melones fault zone, whatever its detailed movement history may be, appears to mark the modified truncated edge of the Palaeozoic orogen in central California. On tectonic grounds, its probable northward extension is viewed as the Trinity fault zone³² (Fig. 1), which, as shown here, truncates the north-east-trending arc and subduction terrains of the eastern Klamath Mountains and abruptly juxtaposes them against arcuate, northerly trending terrains to the west.

The missing pieces

Tectonic elements presumably missing from the Palaeozoic orogen are the south-western continuation of the marginal basin and the remainder of the Devonian-Permian arc complex. I assume that these missing pieces had some finite areal extent and might be recognised in some other geological setting.

Is the western Klamath terrain a marginal basin fragment? The western Palaeozoic and Triassic belt of the Klamath Mountains³⁵ (13, Fig. 1), like the Calaveras and Havallah terrains, is underlain largely by deformed chert-argillite, mafic volcanic rocks and lenses of limestone³⁵⁻³⁷. Large parts of the belt are chaotic^{38,39} and closely resemble diamictite of the Calaveras Formation and Havallah sequence (my observations made in 1972-75). Most fossils from limestone lenses within the southern part of the terrain indicate Pennsylvanian to Permian ages³⁶, although rare Devonian and Triassic fossils have been reported^{36,40}. A probable southern continuation of the Klamath terrain occurs, in addition, west of the Melones fault zone in the north-western Sierra Nevada^{15,32}.

Rocks of the western Palaeozoic and Triassic belt have been interpreted as part of a late Palaeozoic subduction complex related to the late Palaeozoic arc of the eastern Klamath Mountains and northern Sierra Nevada^{2,4,5}, invoking blueschists in two areas near the eastern edge of the belt^{4,5,11,37} (14, 15, Fig. 1). For the following reasons this interpretation is considered untenable.

(1) As argued earlier, the probable north-east-trending

subduction complex related to the late Palaeozoic arc (1, Fig. 1) lies in the north-western part of the eastern Klamath Mountains. (2) The structural trends of the western Palaeozoic and Triassic belt and its southern continuation parallel the Trinity and Melones fault zones which mark the truncated edge of the orogen and are highly discordant with north-east-trending arc terrains. (3) Available evidence indicates that deformation and metamorphism of the sedimentary rocks was post-Permian and pre-late Jurassic^{39,41}. (4) Blueschists in the eastern part of the western Palaeozoic and Triassic belt have yielded a 215-Myr K-Ar date⁵, which allowing for uncertainties in the Triassic part of the time scale⁴², could be as young as late Triassic. These blueschists are clearly part of an inner, early Mesozoic blueschist belt in the Cordillera, including localities in central British Columbia⁴³, the Northern Cascades⁴⁴, east-central Oregon⁴⁵ (16, Fig. 1), and undated lawsonite blueschist along the Melones fault zone in the northern Sierra Nevada (ref. 54) (17, Fig. 1). Published K-Ar ages on blueschists in this belt all cluster around 210-220 Myr^{5,43,44}. This and the geological setting of these blueschists strongly indicates that they formed in subduction zones related to Upper Triassic and Lower Jurassic arc rocks in eastern California, western Nevada, Idaho and British Columbia^{42,47-49}.

I believe that the rocks of the western Palaeozoic and Triassic belt are best interpreted as part of an already chaotic terrain that originated elsewhere and may have been subducted during the early Mesozoic with the local production of blueschist. I suggest (Fig. 2) that the western Palaeozoic and Triassic belt represents part of the missing marginal basin sequence and has been shifted some 500-600 km north-west from its original position opposite the Calaveras Formation after the truncation event. This interpretation accounts for the fact that the apparently correlative rocks of the Calaveras Formation and western Palaeozoic and Triassic belt lie on opposite sides of the Trinity-Melones fault zones (Fig. 1), and also allows for the existence of blueschist in the Klamath terrain and its southern continuation and its apparent absence in the Calaveras Formation^{13,14,54} (also my unpublished data).

Andesitic rocks flanked on both sides by ophiolitic rocks, recently noted in the western Palaeozoic and Triassic belt³⁶, may represent part of a remnant arc which became isolated by continued spreading within the marginal basin. Volcanic rocks within parts of the terrain in Oregon and the north-western Sierra Nevada³⁰ may also represent parts of remnant arcs, and shreds of ophiolites are probably represented in these areas as well.

The north-north-west-trending central metamorphic belt (18, Fig. 1), a tectonic sliver of hornblende schist and overlying mica schist^{37,51} between the western Palaeozoic and Triassic belt and the Trinity fault zone, has late Devonian to Mississippian K-Ar and Devonian Rb-Sr ages⁴¹. Burchfiel and Davis^{4,5} regard this terrain as the subduction complex related to Devonian arc activity to the east although blueschists are lacking, and the Trinity fault zone juxtaposes the central metamorphic belt directly against the south-western part of the north-east-trending arc complex (Fig. 1). It could alternatively be an exotic fragment², but as Burchfiel and Davis^{4,5} point out, its metamorphic ages suggest some relation to the Antler orogeny. A highly speculative possibility, adopted in Fig. 3, is that rocks of the central metamorphic belt represent a deep-seated southerly portion of the Antler orogenic belt rifted away from its original position together with rocks of the western Palaeozoic and Triassic belt. The westward vergence of synmetamorphic overturned folds within the terrain^{4,5,51}, however, is difficult to explain unless it is attributed to eastward subduction beneath the Antler orogenic belt^{5,12}. Unfortunately, blueschists are lacking. Whatever its origin, the central metamorphic belt now forms a thrust plate that has overridden the western Palaeo-

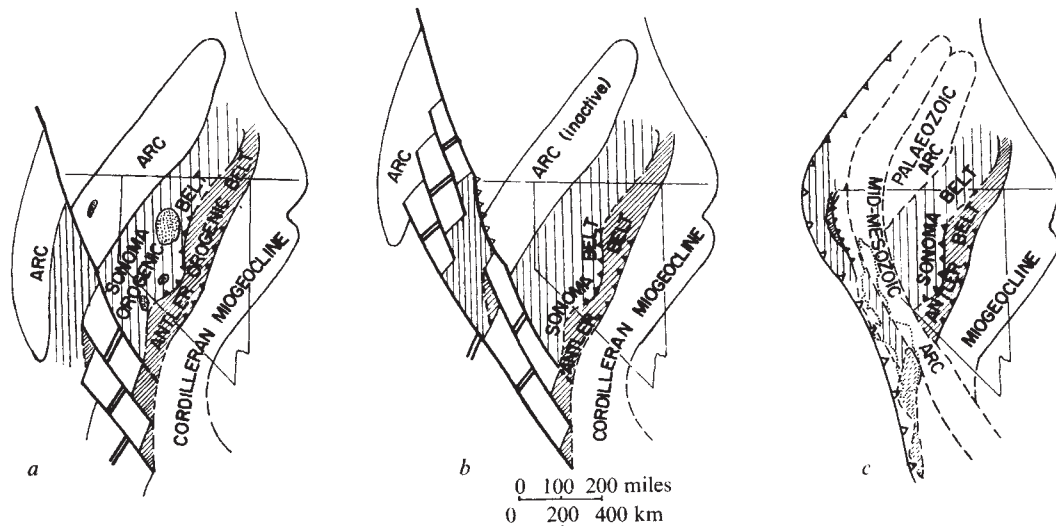


Fig. 3 Tectonic diagrams showing proposed sequence of events leading to present distribution of Palaeozoic terrains in the Cordilleran orogen. *a*, Stage of fragmentation during early Triassic. Stipple pattern shows areas of Permo-Triassic silicic volcanism. *b*, Hypothetical stage of fragmentation during middle Triassic. Arc and marginal basin fragment are separated by jump in plate boundary; marginal basin fragment begins to

underthrust truncated edge of arc. *c*, Initiation of subduction along truncated margin during the late Triassic. Partial subduction of marginal basin fragment produces local blueschist metamorphism in Klamath Mountains and Northern Sierra, Nevada. Late Triassic to mid-Jurassic arc is superimposed on older tectonic elements. The dot pattern shows future site of Upper Jurassic–Upper Cretaceous Sierra Nevada batholith.

zoic and Triassic belt for 20–30 km, and has itself been overridden by rocks of the eastern Klamath belt for distances of 30 km or more¹¹.

I next consider a possible arc fragment in the Pacific North-West. Figure 2 depicts the original south-western extent of the late Palaeozoic volcanic arc and marginal basin which fringed North America before the truncation event. The western Palaeozoic and Triassic belt and its Sierran continuation are shown as the south-westerly part of the marginal basin. Local remnant arcs are also shown.

The remaining part of the orogen to be identified is the corresponding arc segment which must originally have lain along the north-western flank of the marginal basin. Monger and Ross⁷ have suggested that Upper Palaeozoic rocks of the Coast Mountains of British Columbia and south-eastern Alaska may be far-travelled from their original sites of accumulation. Jones and others⁸ argued that the Lower Palaeozoic rocks of south-eastern Alaska, at least, were derived from the truncated margin of the Cordilleran orogen, probably in northern California. Palaeozoic sequences containing volcanic rocks of basaltic to rhyolitic composition occur widely within this 1,500-km-long region and rest on a basement of metavolcanic and ultramafic rocks^{6,9}.

It seems likely that the Palaeozoic rocks in this region in part represent a volcanic arc complex that developed on lower Palaeozoic basement in late Palaeozoic time⁷. Much of the terrain, especially its northern part, can be viewed as the missing arc segment required by the arguments presented here, in turn satisfying the palaeontological constraints invoked by Monger and Ross⁷. If so, the puzzle is relatively complete (Fig. 2).

Possible sequence of fragmentation

Figure 3 shows hypothetical stages of fragmentation of the orogen, resulting in dispersal of its component parts. Figure 3*a* shows the initiation of separation, which perhaps began in the early Triassic, after the Sonoma orogeny^{4,5}, when silicic volcanism was widespread within the orogen. Figure 3*a* shows a rhombochasm, like the Gulf of California, forming behind the arc and marginal basin fragment.

Figure 3*b*, approximately middle Triassic, shows two

significant changes: a second rhombochasm begins to separate the arc segment from the Palaeozoic marginal basin fragment. There is no direct evidence of timing, but some such mechanism is required for the separation of the arc and marginal basin fragments. The marginal basin fragment perhaps impinged obliquely against the blunt end of the arc thus retarding its motion, and producing thrusting along its boundary.

Figure 3*c*, drawn for late Triassic to early Jurassic time, shows that the marginal basin fragment has underthrust the truncated end of the arc with the initiation of north-eastward subduction of lithosphere beneath the newly reshaped continental margin. This subduction resulted in the production of blueschists and the generation of a new magmatic arc at a high angle to the Palaeozoic trends^{4,5,15,46}. The arc fragment has by this time progressed on its way to south-eastern Alaska.

Implications and conclusions

The model described here has several attractive features. It provides a plausible context for assembly of the various Palaeozoic terrains of the Klamath Mountains, one that seems more satisfactory than repeated collisions of exotic plates²; it is consistent with known ages of deformation and metamorphism, and it provides some insights into the basic similarities and differences between Palaeozoic terrains in the Klamath Mountains and the western Sierra Nevada of California. In addition, it is consistent with tectonic inferences drawn from studies of the Canadian and Alaskan Cordillera^{6,7}.

If this model has any validity, it seems to imply that western North America was repeatedly subjected to major north-westerly transcurrent movements, beginning about 850 Myr ago, with north-west-south-east rifting which initiated the Cordilleran geosyncline⁵², continuing with the early Mesozoic movements suggested here, and again in the Cainozoic, with slicing and north-west tectonic transport related to the San Andreas system.

Because chaotic sequences like the Calaveras, the western Palaeozoic and Triassic belt, and the Havallah sequence may have acquired their chaotic character behind island arcs, with or without subduction, it seems that the chaotic character of assemblages alone may not be sufficient for

them to be labelled subduction complexes; other lines of evidence are needed.

The proposed scheme of reassembly and fragmentation is consistent with many known aspects of Cordilleran geology, but is obviously an oversimplification of complex, controversial and as yet poorly known geology. I consider the first part of this article, which locates the truncated edge of the orogen, the most certain. The second and third parts are successively more speculative, and new data will require modification or even rejection of some of the suggestions. I present this analysis as an alternative to the orthodox models of orthogonal subduction and collision tectonics currently in vogue^{2,4,5,9}. As noted here, major transcurrent motions along longitudinal faults deserve as much careful consideration as simple subduction in the generation of viable models of the tectonic evolution of Cordilleran mountain belts.

I thank W. Alvarez, D. S. Cowan, I. W. D. Dalziel and W. R. Dickinson for reading the manuscript, and D. S. Cowan and W. R. Dickinson for discussions. This work was partially supported by the Department of Geological Sciences, Columbia University.

Received October 16, 1975; accepted February 26, 1976.

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letters to nature

Observational tests in cosmology

It is often said that the determination of the deceleration term is one of the main aims of observational cosmology. In the following comments this view is questioned, and in a universe of indeterminate density, the third time derivative of the scaling variable is shown to be of greater fundamental importance.

Let $K (= k/R^2)$ be the curvature of a homogeneous and isotropic model of the Universe, where R is the scaling variable, and k is the curvature constant indicating whether the model is closed ($k = 1$) and space is finite, or open ($k = 0, -1$) and space is infinite. According to general relativity, the dynamic equation of a zero-pressure model is

$$K = 4\pi G\rho - H^2(q+1) \quad (1)$$

where ρ is the average mass density. The Hubble term H and the deceleration term q are

$$H = \dot{R}/R$$

$$q = -\ddot{R}/RH^2$$

and dots denote time derivatives. The cosmological term, which at present cannot be measured independently by any known means, is

$$\Lambda = 4\pi G\rho - 3qH^2 \quad (2)$$

The verification of the important cosmological equation (1)

requires measurements of the quantities K , ρ , H , and q . When, however, (1) is assumed to be valid, the resolution of the issues as to whether the Universe is open or closed, and whether the Λ term is zero or not, requires measurements of ρ , H , and q .

In principle the quantities K , H , and q can be determined, although in practice their precise determination is exceedingly difficult¹. The determination of the average density ρ is quite another matter—for its exact value cannot be determined, even in principle. The reason for this is obvious. We know approximately the contribution to the average density from luminous galaxies, and can even attempt with tongue in cheek to estimate the contributions from the intergalactic medium and known non-luminous objects such as stellar black holes. But we cannot, of course, include all the unknown contributions (dead galaxies? primordial black holes? . . . ?) that will be discovered in the future.

Cosmological tests of a local nature² concerning the sign of K and the value of ρ , based on age determinations, assume first the validity of (1) and second a zero value for the Λ term, and they must therefore be regarded as doubly uncertain.

If in principle the density is indeterminate, then, like the Λ term, it must be removed from (1). In a form analogous to the deceleration term, let

$$Q = \ddot{\ddot{R}}/RH^3$$

be a dimensionless third-order time derivative. The differentiation of equation (1), with ρR^3 kept constant, gives

$$K = H^2(Q-1) \quad (3)$$

with the auxiliary relationships

$$\Lambda = H^2(Q-2q) \quad (4)$$

$$4\pi G\rho = H^2(Q+q) \quad (5)$$

Thus the removal of ρ also eliminates q from the cosmological equation (3), and the deceleration term is reduced to a subordinate role in equations (4) and (5).

From equation (3) it is seen that verification of general relativity on cosmic scales requires measurements of K , H , and Q , and the Universe is closed (open) when Q is $>$ ($<$) 1. Weinberg³ has drawn attention to the problem of extracting separately the values of K and q from observational data. Attention is now drawn to an additional problem: until the density is known with greater precision than is possible at present, the value of q is irrelevant for testing the validity of general relativity. Moreover, when general relativity is assumed to be valid on cosmic scales, the traditional observational parameters H and q are inadequate for deciding such questions as: (1) is the Universe open or closed? (2) is the Λ term zero? and (3) is there 'missing mass'? Verification, and the resolution of these issues, depends on the value of Q and therefore $\ddot{R}$, and at present, unfortunately, we have no knowledge of this value.

The assumption that the Λ term is zero greatly simplifies the problems of observational cosmology. In this case, $Q = 2q$, and

$$K = H^2(2q-1) \quad (6)$$

and verification requires measurements of K , H , and q , and the Universe is closed (open) when q is $>$ ($<$) 0.5.

Reasons for rejecting or retaining the Λ term are still of a philosophical flavour. A particularly appealing argument proposes that Λ is retained provisionally as a reminder of the possibility of undiscovered cosmic complexity. As an illustration, the Universe consists perhaps mainly of matter and relatively little antimatter⁴. Does this cosmic predilection for matter arise from some quirk in the properties of elementary particles, or does it come in some unknown way from the Λ term and hidden richness in the properties of space-time?

Of the physical systems that range in size from atoms ($\sim 10^{-8}$ cm) to galaxies ($\sim 10^{23}$ cm), electromagnetic interactions dominate on the atomic scale at one extreme, and gravitational interactions dominate on the galactic scale at the other extreme. It is found, on going down five orders of magnitude in size from atoms to elementary particles, that electromagnetic theory is inadequate and is supplemented by weak and strong interactions. Why then, in the absence of observational proof, should we assume that gravitational theory remains unmodified on going up five orders of magnitude in size from galaxies to the Universe? A universe that contains everything is surely as complex as an elementary particle? Considerations of this kind again suggest that the Λ term should be provisionally retained, even though at present we are not aware of its purpose in the cosmic design.

Most observational work in general relativity has in the past been devoted to testing the Einstein equation within stellar-like systems. But we must not forget that confirmation of general relativity in the Solar System and on the scale of stellar systems does not automatically guarantee that the theory is unassailable on the cosmic scale. The Riemannian metric is the most appealing feature of general relativity when applied to cosmology. The identification, however, of the coupled 'matter' tensor solely with energy and momentum has not been submitted to tests on the cosmic scale, and here again is reason for retaining the Λ term until verification is forthcoming. On this subject it is worth recalling Einstein's⁵ words: "The right side [the 'matter' tensor] is a formal condensation of all things whose comprehension in the sense of a field theory is still problematic. Not for a moment, of course, did I doubt that this formulation was merely a makeshift in order to give the general principle of relativity a preliminary closed form."

I acknowledge the hospitality of the National Radio Astronomy Observatory operated by Associated Universities Inc., under contract with the National Science Foundation.

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Received February 17; accepted March 3, 1976.

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Evidence for a 17-d periodicity from Cyg X-3

Cyg X-3 (3U2030+40) has exhibited phenomena which are observationally unique among identified X-ray sources. The giant radio flare of 1972 is, perhaps, the most spectacular such anomaly, but there are others no less unusual. A wide variation in X-ray spectra has been observed, including the identification of X-ray emission lines at some times, and consistency with a black body at others¹. The approximately sinusoidal 4.8-h variation² is at a period far in excess of any rotation period which has been ascribed to the compact members of other binary sources, and at least four times shorter than any comparable orbital period. Models have been constructed which identify the 4.8-h variation with orbital period³⁻⁵, acknowledging the peculiar geometries which could give rise to a smooth 4.8-h effect in observed X rays. We here present data indicating that a much longer periodicity of ~ 17 d is also characteristic of Cyg X-3.

The Ariel-V All-Sky Monitor (ASM), from which most of our data are taken, has been described in detail elsewhere⁶. The

Fig. 1 Approximately 100 d of single-orbit Cyg X-3 and Cyg X-1 ASM data obtained between December 1974 and March 1975 folded at the Cyg X-3 period (with indicated phase) of ref. 7. The indicated χ^2 values are for 10-bin folds (9 degrees of freedom) against the hypothesis of a constant source intensity.

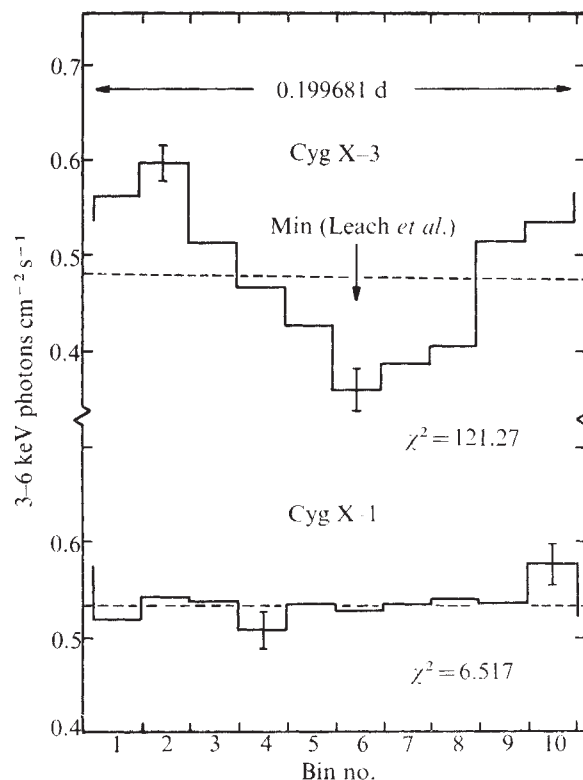
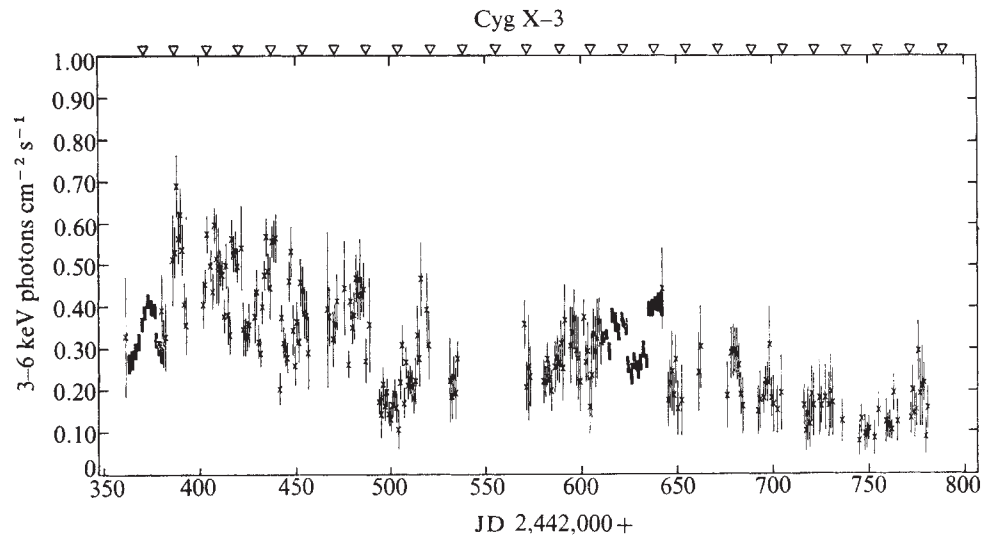


Fig. 2 Continuous record of Cyg X-3. The data points are daily averages of ASM data, with $\pm 1\sigma$ error bars. The solid bars are $\pm 1\sigma$ thick, 1.6-d averages of SSE data. The latter are 2–18 keV measurements which are normalised to the natural ASM ordinate with the response of both instruments to the Crab Nebula. The grid above the data indicates the positions of expected maxima with the best fold values of period (16.75 d) and maximum (JD 2,442,522).



important parameters are an effective pinhole area of 0.6 cm^2 in the energy band 3–6 keV, an average duty cycle for source observation of $\sim 1\%$, and no temporal resolution finer than 100 min. Typically, ~ 10 Cyg X-3 counts are accumulated each 100-min orbit in a resolution element of spatial dimensions $\sim 10^\circ \times 10^\circ$, with a background of ~ 2 counts.

Figure 1 is a useful verification of experiment performance on Cyg X-3. Single-orbit data from ~ 100 d are plotted [4.8 h] from Cyg X-3 and, as a control, Cyg X-1 (with which it might conceivably be confused with resolution elements centred $\sim 10^\circ$ apart). Data points are accepted only if they represent an unambiguous determination of source intensity (the possible contribution from other sources is $< 10\%$, and the intensity is at least twice the estimated 1σ error after all corrections have been applied). In folding, the data from each 100-min accumulation are tagged with the orbit midtime. Each bin in Fig. 1 is statistically independent of the others, as an orbit contributes only to the bin that contains its midtime (even though the data are accumulated over the equivalent of 3–4 bins). Both the shape (and phase) are in excellent agreement with the results of ref. 7, indicating that the present measurements are consistent with their period (and error) of 0.1996811 ± 0.0000016 d.

Additionally there is considerable variability in the day-to-day intensity of Cyg X-3, as shown in Fig. 2. This behaviour is in marked contrast to the constant (within the relatively poor statistics) day to day nature of low-state Cyg X-1 data measured simultaneously with the same experiment⁸. There is, however, some indication of regularity in the Cyg X-3 variations on time scales ≥ 1 week. As the Cyg X-3 data from individual orbits do not always satisfy the 2σ condition, the ASM data used in Fig. 2 are derived from $\sim \frac{1}{2}$ -d accumulations which are then analysed in exactly the same way as are the individual orbits (they are treated as single long duration orbits). It is not possible unambiguously to compensate completely for the 4.8-h variation, so no attempt has been made to do so. This variation, as well as gaps in the finite data string and an apparently erratic source behaviour, result in many periods in excess of a few days which give relative χ^2 maxima. The effect near 17 d is not only the most significant statistically, but also exhibits a roughly symmetrical χ^2 distribution which has a width commensurate with the length of the data sample. For more than one year's data folded in this way, the best period is 16.75 ± 0.25 d with a phase at maximum of JD 2,442,522 ± 2 (as determined from the folded data, not an individual peak) near the midpoint of the data accumulation interval.

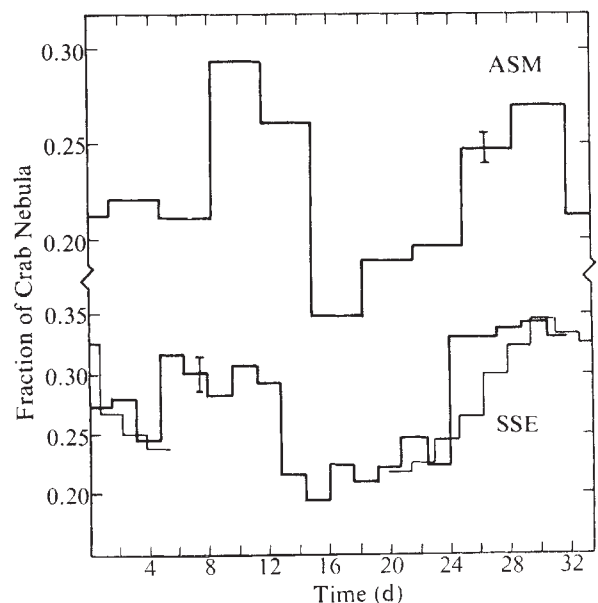
Figure 2 also contains data from the Ariel V Sky Survey Experiment (SSE) against which the 17-d hypothesis may be tested. It is important to note that the latter measurements are obtained in the gaps of the ASM coverage, as the two experiments possess mutually exclusive fields of view. The data are generally consistent with the displayed grid of 16.75 d, but it is

clear that the effect is not completely reproducible (as the apparent maxima are not always clearly defined, and are sometimes absent). Nevertheless, the general agreement of the SSE data with the ASM grid is independent evidence for the reality of the candidate period.

Figure 3 contains the same ASM data folded [33.5 d], or precisely twice the best value of period. The lower trace is the SSE data plotted in phase with the upper trace. Attempts to test the 16.75-d hypothesis with older data in the literature are inconclusive (as these are essentially point measurements), but it is interesting to note that the highest intensity value of Cyg X-3 reported from Uhuru in ref. 7 is in phase with the ASM maxima.

In ref. 1, the authors pointed out that the "high intensity state" of Cyg X-3 is relatively well fitted by a structureless black body, in contrast to the considerably more complex spectra observed in lower intensity states. They further suggest that the total source luminosity is close to the Eddington limit, and approximately constant, regardless of spectral form. This interpretation of the "high intensity state" is, therefore, a manifestation of the relatively better efficiency of contemporary experiments near the black-body peak than at higher energies. We are assuming here that this interpretation is correct, and

Fig. 3 ASM data for > 1 yr folded at 33.5 d (upper trace), and SSE data plotted in phase (lower trace). Both are normalised with their respective responses to the Crab Nebula.



that the times of Cyg X-3 maximum correspond to increased electron scattering in the source.

One possible explanation would arise naturally if the 17-d effect was the orbital period of the binary system containing Cyg X-3. In this case, however, the stability of the 4.8-h variation (which would now be interpreted as a slow source rotation) would seem to constrain this hypothesis severely. No apparent 17-d Doppler variation in the 4.8-h modulation is detectable in our data ($v_x \sin i < 300 \text{ km s}^{-1}$) and it is difficult to account for the long term stability of this period unless the surface field is much lower than that expected if the observed 4.8-h modulation arises from rotation.

A less drastic suggestion (one which does not alter the identification of 4.8 h with the orbital period) is that the 17-d effect is analogous to the 35-d variation in Her X-1 (see ref. 9). The consistency of both Cyg X-3 and Her X-1 with contact-binary models (in contrast to the supergiant–stellar-wind models reconcilable with the mass source in other identified X-ray binaries) makes this conjecture attractive. It is interesting to note that the interpretation of this effect in terms of free precession^{10,11} does not necessarily require a neutron-star source for Cyg X-3 just because the 17-d and 35-d time scales are comparable. A precession period of 17 d is entirely consistent with either a neutron star with rotation period ~ 1 s, or a white dwarf having a rotation period of the order of minutes. Its interpretation in terms of the precession of the primary member of the binary system (compare ref. 12) is likewise insensitive to the nature of the secondary. While it does not appear that the 17-d effect can unambiguously distinguish between a white dwarf and a neutron star, the inferred high luminosity and relatively hard spectrum out to $\sim 40 \text{ keV}$ would seem to favour the latter.

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Satellite observations of planetary waves in the mesosphere

THE pressure modulator radiometer (PMR) is one of the instruments carried by the Nimbus 6 observatory satellite launched on June 12, 1975 into a near polar circular orbit at an altitude of $\sim 1,000 \text{ km}$. The PMR sounds atmospheric temperature in the upper stratosphere and mesosphere (the 40–95 km height range) by measuring infrared emission from atmospheric carbon dioxide in its $15\text{-}\mu\text{m}$

band¹. With this instrument measurements of mesospheric temperature with global coverage are being made for the first time. Here we present some of the first results from the PMR, which show, in particular, the propagation of a planetary wave from the troposphere up to the mesopause.

The radiation to be detected is passed through a cell of carbon dioxide which acts as a filter and in which the cell pressure is varied sinusoidally at $\sim 15 \text{ Hz}$. The amplitude of the resulting signal selected at this frequency is a measure of the intensity of incident radiation from very narrow spectral intervals near each line centre. This radiation originates in the mesosphere, so the PMR provides a method of measuring the temperature at mesospheric levels. This basic technique^{2,3} has been used in the Nimbus 4 and 5 selective chopper radiometers (SCR)^{4,5} which measure emission up to $\sim 55\text{-km}$ altitude by chopping between different cells of carbon dioxide. By varying the pressure in a single cell, problems of mismatch between cells are avoided and temperature can be sounded to much higher levels.

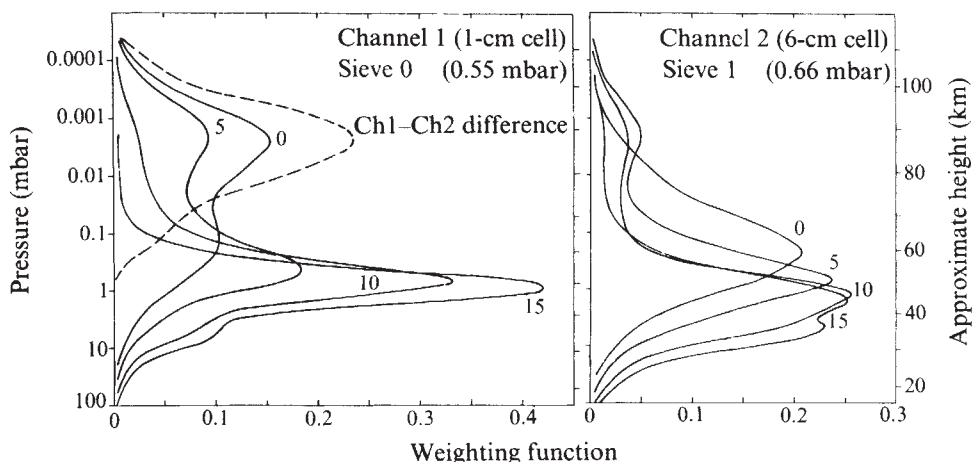
The Nimbus 6 PMR has two modulators called channels 1 and 2 which have cells of path length 1 and 6 cm respectively. Two different methods are used to vary the altitude range being sounded. The first is to vary the mean pressure in a modulator cell between 0.5 mbar and 4 mbar by altering the temperature of some molecular sieve contained in a thimble attached to the cell (and hence the amount of gas adsorbed on it).

The second method uses Doppler scanning. Observing at a few degrees from the nadir along the direction of flight causes a Doppler shift of the incident radiation because of the velocity of the satellite. The CO_2 emission lines from the atmosphere are moved relative to the CO_2 absorption lines in the cell by an amount comparable with the width of the spectral lines in the mesosphere. With this spectral shift the pressure modulators select from narrow spectral intervals in the wings of the lines which originate from lower altitudes. Figure 1 shows a selection of weighting functions (the functions which describe the region of the atmosphere from which the radiation detected by the PMR originates) for different cell pressures and angles of view, showing that the altitude range 40–90 km can be well covered by such a combination. The field of view of the radiometer is 4° (full angle) along the direction of flight and 20° perpendicular to the flight direction, corresponding to a rectangle in the atmosphere of horizontal dimensions $70 \times 350 \text{ km}$. Doppler scanning is arranged in flight by tilting the primary mirror of the optical system so that the direction of view moves from $+15^\circ$ to -15° with respect to the nadir along the direction of flight. One scan takes 88 s, the rate of scan being arranged so that during the scan the same part of atmosphere is being viewed.

The PMR has functioned continuously since June 20, 1975. From each day's data, hemispheric maps have been drawn for each of the channels of the radiance at the centre and end of the scan respectively. Examples are shown in Fig. 2 for the Southern Hemisphere, for August 25, 1975, a day on which there was considerable planetary wave activity. For the lowest level shown ($\sim 45 \text{ km}$) notice the warm region at $\sim 90^\circ$ longitude and 65°S latitude. The wave amplitude is higher at $\sim 50 \text{ km}$ and the warm region has shifted westward to $\sim 15^\circ$ longitude. The westward shift continues with increasing altitudes to $\sim 65 \text{ km}$ but the wave amplitude decreases. Notice that the wave can still be identified at the highest level ($\sim 85 \text{ km}$ altitude). A westward tilt of geopotential height is well known⁶ to be associated with waves in which the energy is propagating upwards. A considerable amplitude of wave number two (two maxima round the pole) is evident at the lower levels, but has not penetrated far into the mesosphere.

The radiance field of the top PMR channel (Fig. 2d) seems at first sight to be surprisingly flat, and does not

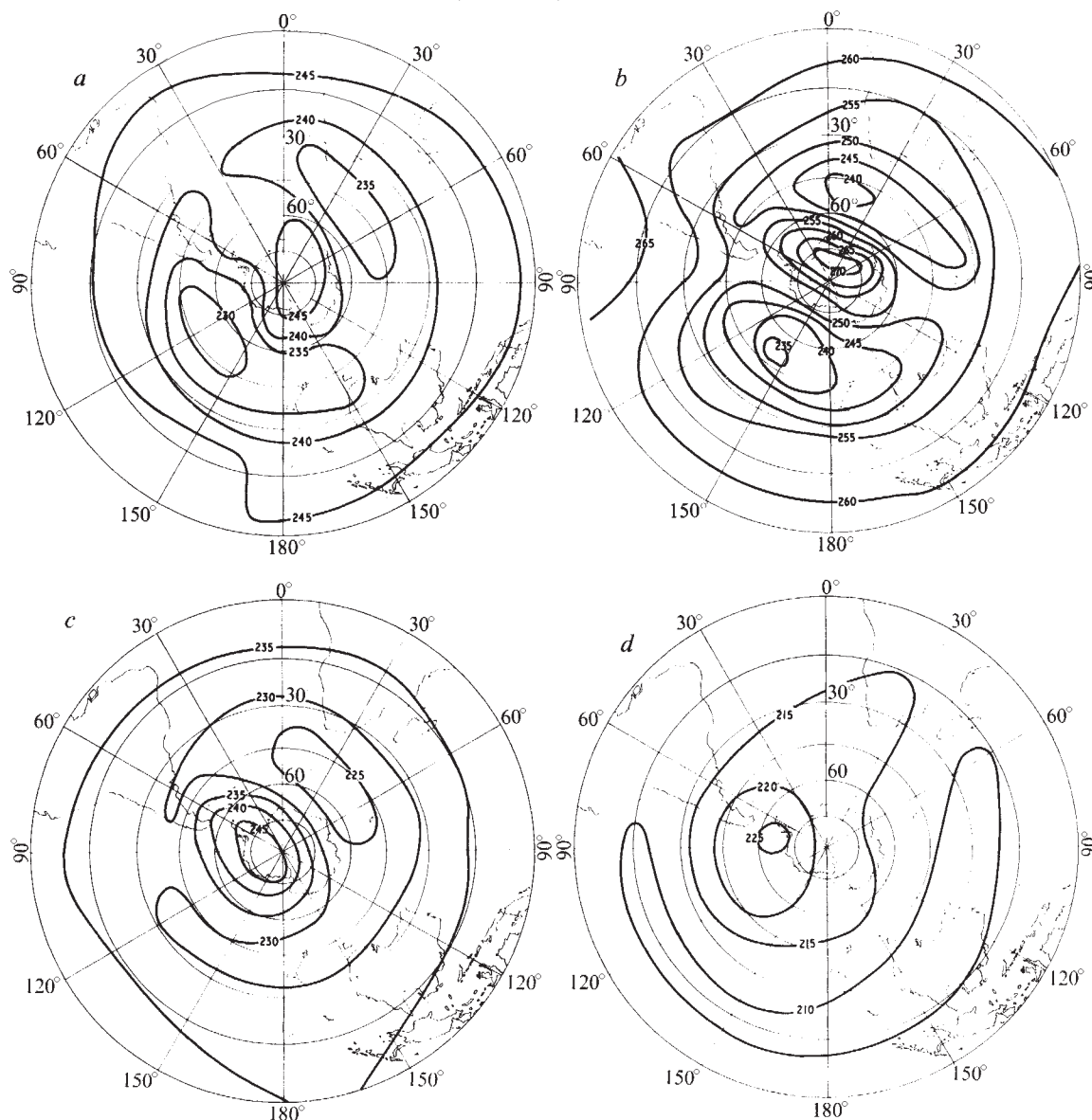
Fig. 1 Weighting functions for the 2 channels of the PMR for their lowest cell pressures for different angles of view from the nadir. The dashed weighting function in *a* is the weighting function applicable to the nadir view for $1.67 \times$ (channel 1) $- 0.67 \times$ (channel 2).



indicate the presence of large amplitude gravity waves often observed by meteorological rockets. This is because the vertical wavelengths of such disturbances are short compared with the vertical averaging of ~ 20 km produced by

the PMR. It is likely also that they possess horizontal distance scales which are short compared with the size of the field of view. The day-to-day consistency of the radiance fields at the top level demonstrates that there is little

Fig. 2 Polar stereographic maps of the Southern Hemisphere radiance (expressed as equivalent temperature) for August 24, 1975 for *a*, channel 2, 0.66 mbar, 15° from nadir; *b*, channel 1, 0.55 mbar, 15° from nadir; *c*, channel 2, 0.66 mbar, nadir view; *d*, $1.67 \times$ (channel 1) $- 0.67 \times$ (channel 2) nadir view, cell pressures as in *a-c*. The appropriate weighting functions are shown in Fig. 1; they peak at ~ 40 km (*a*), 48 km (*b*), 61 km (*c*), 87 km (*d*).



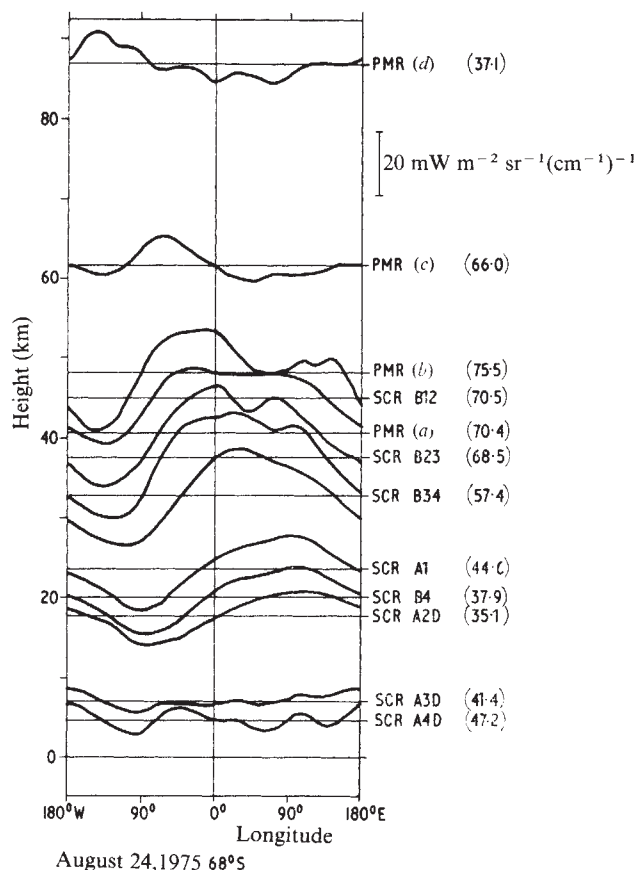


Fig. 3 Height-longitude cross section around the 68° S latitude circle for August 24, 1975. For each PMR and SCR channel the mean radiance is shown in brackets near the centre of the weighting function peak, and the deviation from the mean radiance is plotted as the ordinate at each of the levels. The PMR channels are labelled (a), (b), (c) and (d) corresponding to the key of Fig. 2.

residual atmospheric noise left after this averaging process.

Figure 3 gives more details of the wave. The PMR and Nimbus 5 SCR (which has functioned continuously for 3 yr) are in excellent agreement in the region where they overlap. The same planetary wave can be identified from the troposphere up to the mesopause, over which altitude range it shows a consistent westward tilt with altitude—the longitude of the maximum changing by nearly 360° from the bottom to the top.

Construction of the PMR involved a large number of personnel at Oxford University, the Rutherford Laboratory, Marconi's Space and Weapons Laboratory at Frimley, and the Department of Applied Physics, Reading University. The project was supported by the SRC. We also acknowledge the cooperation of the Nimbus Project, NASA.

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Ball lightning

A DRAMATIC and characteristic ball lightning event, important because it may reveal details of the energetics of the phenomenon, was reported by a housewife to have occurred during a vigorous thunderstorm in the Midlands area of England on August 8, 1975. During this storm, a number of houses in the area were struck by lightning.

The witness was in the kitchen of her house in Smethwick, Warley, at about 1945, when a sphere of light appeared over the cooker. The ball was ~10 cm across and surrounded by a flame-coloured halo; its colour was bright blue to purple. The ball moved straight towards the witness at an estimated height of 95 cm from the ground. Burning heat was felt, and there was a singeing smell. A sound something like a rattle was heard. The ball was in sight for only a second or so because its lifetime was cut short:

"The ball seemed to hit me below the belt, as it were, and I automatically brushed it from me and it just disappeared. Where I brushed it away there appeared a redness



Fig. 1 Part of the dress alleged to have been damaged by "ball lightning", showing area and extent of damage. Scale: average diameter of spots in pattern is 19 mm.

and swelling on my left hand. It seemed as if my gold wedding ring was burning into my finger."

Where the ball struck her, the clothing of the witness was damaged. A hole was produced in her dress and her tights. Her legs were not actually burned, however, but became red and numb. The dress has been secured for examination, and tests are currently being carried out to try to establish the nature of the damage. There is a central area (Fig. 1) where the synthetic fibre of the dress has disappeared altogether, and the shape of the hole is in agreement with the witness' statement that she brushed the ball downward a couple of inches. The hole is ~11×4 cm. Around its perimeter, the material has shrivelled, but is not charred. In addition, there is a secondary area where the fibre has shrivelled, and the pattern printed on the fabric has faded noticeably. The position of the primary damage is ~20 cm from the hem of the dress, so it is estimated that the ball struck at ~95 cm from the ground.

Although thunder and lightning were going on at the time, the witness is not sure whether or not the manifestation was associated with a thunderclap. The ball exploded with a bang, but she is not certain whether it exploded at

the exact time at which she touched it; she felt this to be the case, however. Before my contacting her, the witness had had no acquaintance with the phenomenon of ball lightning.

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Possible relationship between soil fulvic acid and polymaleic acid

In the course of structural investigations into the organic matter component of soil, it became clear that the fulvic acid solutions¹ (the alkali-soluble fractions of humus not precipitated by acid) contained a high proportion of their soluble organic matter as a polymer, consisting of a carbon skeleton highly substituted by carboxyl groups. The solutions also normally contained peptide and polysaccharide mixtures or complexes which could be removed almost entirely by fractionation on charcoal², a technique which allowed the isolation of the polycarboxylic acid. Brown acidic polymers virtually identical with those found in the fulvic acid solution were recovered from podzol B_h horizon humus by extraction of the soil with dilute mineral acid or trisodium citrate solutions (pH 7.0) followed by dialysis.

Infrared spectroscopy, elemental analysis and a study of the degradation products of these polymers and their derivatives suggested that the polymers had an aliphatic or alicyclic backbone substituted predominantly by vicinal carboxyl groups. The soil polymers still contained polysaccharide, phenolic derivatives and nitrogen, but in low amounts, the last being accountable as amino acid residues and the ammonium ion. The product arising from the hydrolysis of pyridine-catalysed homopolymerised maleic anhydride³ was found to be chemically and structurally related to the natural polymers, as shown by the similarities between their infrared absorption spectra (Fig. 1), their analysis (polymaleic acid: C 48.76, H 3.30, N 0.82%; B_h citrate-soluble humus: C 47.62, H 3.39, N 0.86%; for comparison, fulvic acid polymer: C 46.18, H 2.68, N 0.95%) and certain of their acid-hydrolysis products (Fig. 2). The dissimilar hydrolysis products, levulinic acid and the phenolic acids, could arise from species adsorbed on to or incorporated into the natural polymer, and this was demonstrated with the synthetic polymer. Slight differences between the potentiometric titration curve of the polymaleic acid⁴ and that of the soil polymer were similarly explicable, while the solution-pH visible absorption spectra relationships were identical.

Fig. 1 Infrared spectra of *a*, polymaleic acid, *b*, fulvic acid in KBr pressed disks (1-mg sample and 170-mg KBr in a 12-mm diameter disk). Absorption in (*b*) near 1,000–1,100 cm⁻¹ is from carbohydrate.

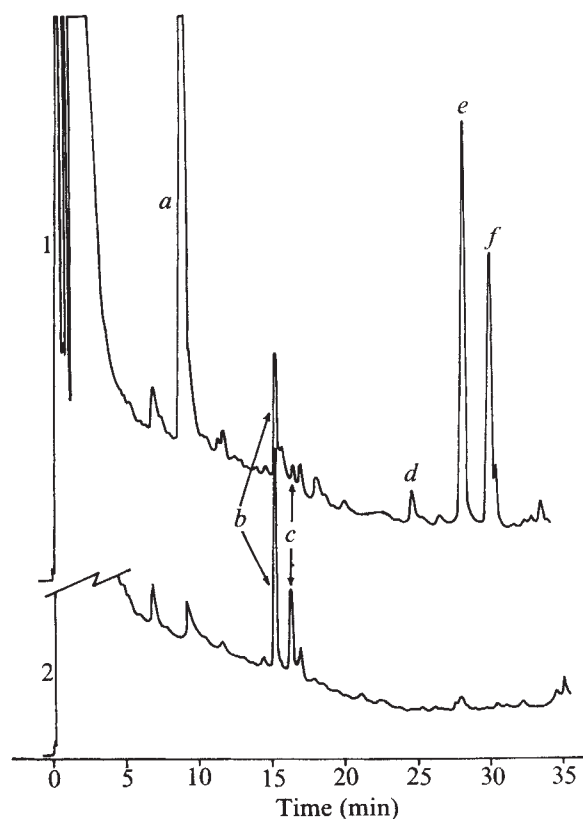
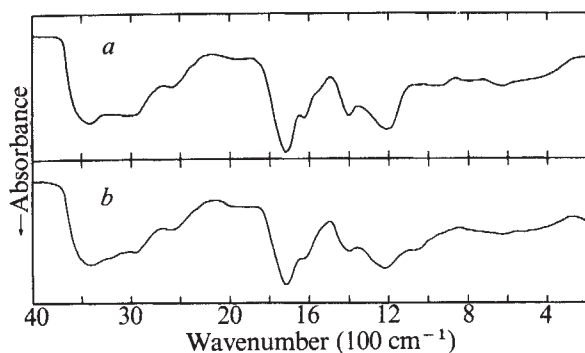


Fig. 2 Gas chromatography curves of trimethylsilyl derivatives of ether-soluble products from 6 M HCl hydrolysis of (1) Humus podzol B_h citrate-soluble humus, (2) Polymaleic acid. Products identified by gas chromatography-mass spectrometry: *a*, Levulinic acid TMS; *b*, Succinic acid di-TMS; *c*, Fumaric acid di-TMS; *d*, 4-Hydroxybenzoic acid di-TMS; *e*, Vanillic acid di-TMS; *f*, 3,4-Dihydroxybenzoic acid tri-TMS. GC conditions: 3% OV-1 on AW silanised Diatomite C (1.5 × 0.006 m); 15 cm³ min⁻¹ nitrogen carrier gas; temperature programme 100 °C isothermal for 5 min, 4 °C min⁻¹ to 250 °C, isothermal at 250 °C for 10 min.

Although the complete structure of polymaleic acid has yet to be elucidated, certain aspects of the structure are known, for example, the existence of vicinal carboxyl groups readily dehydrating to yield anhydride, a property shown spectroscopically by fulvic acid, and unsaturation which accounts for an absorption band near 1,620 cm⁻¹ in the infrared spectrum of fulvic acid. It is these features that make this polymer attractive as a model for fulvic acid. Structures already proposed⁵ for fulvic acid are based on aromatic residues that have been assumed to arise partly from lignin- and/or tannin-derived material. Previous unpublished work by H.A.A. and A. Hepburn has shown that the yields of aromatic molecules from degradations are sufficiently low for these compounds to be considered of minor importance in the structure. Further structural investigations of fulvic and polymaleic acids are in progress.

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Hyperbolic umbilic catastrophe in crystal fracture

WE have demonstrated the existence of a cusp catastrophe in a symmetry-breaking mechanical instability of a close packed atomic lattice^{1,2}, and in an extension of this work we demonstrate here the existence of the more complex hyperbolic umbilic catastrophe³⁻⁵ in a three-dimensional failure-stress locus.

The original cusp was associated with an unstable symmetric point of bifurcation⁶ encountered by a close packed crystal with Lennard-Jones interatomic potentials subjected to a uniaxial tensile stress σ_{11} . Under this stress the lattice follows a primary equilibrium path involving a direct strain ϵ_{11} , and we showed that this path becomes unstable at the critical point A of Fig. 1. At this bifurcation point an unexpected symmetry-breaking shearing strain ϵ_{12} would develop explosively in the presence of even an infinitesimal disturbance, leading to a violent fracture of the crystal.

In this situation a corresponding small shearing stress σ_{12} acts as an 'imperfection' rounding off the bifurcation as shown by the lighter lines and giving rise to a sharp two-thirds power

Fig. 1 The upper picture shows the equilibrium paths of the atomic lattice when $\sigma_{22} = 0$, while the lower left-hand picture shows the projection of these paths into the $(\sigma_{11}, \epsilon_{12})$ plane where we see the well known unstable-symmetric point of bifurcation or cusp catastrophe⁶. In general, stable regions of paths are drawn as solid lines while unstable regions of paths are drawn as broken lines. The lower right-hand picture shows the corresponding stability or catastrophe boundary as a failure-stress locus with the predicted two-thirds power law sensitivity.

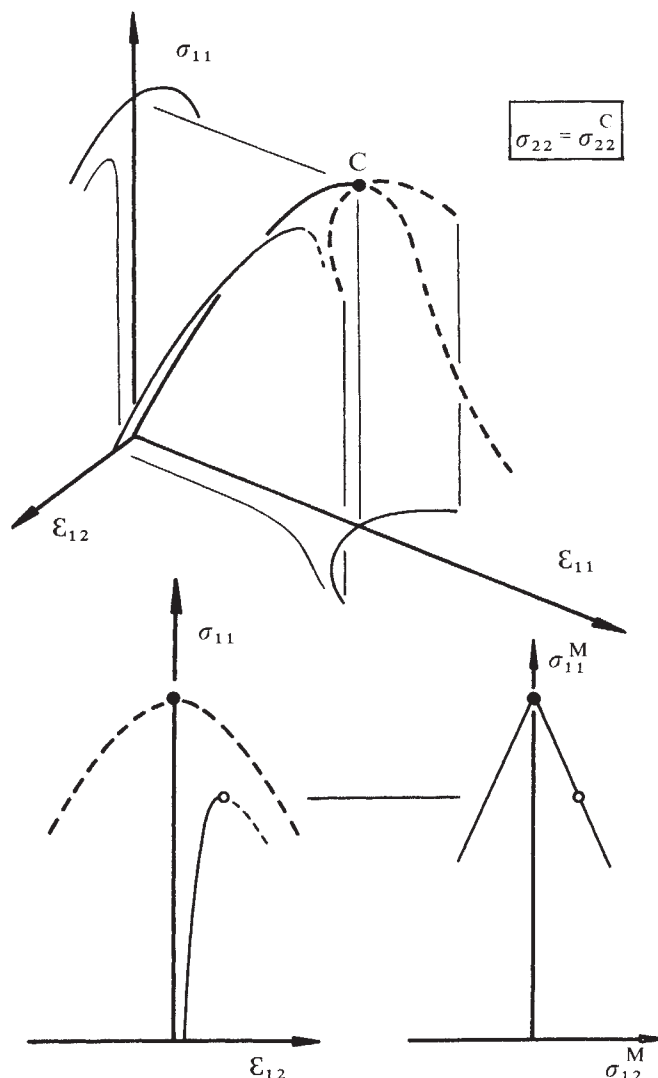
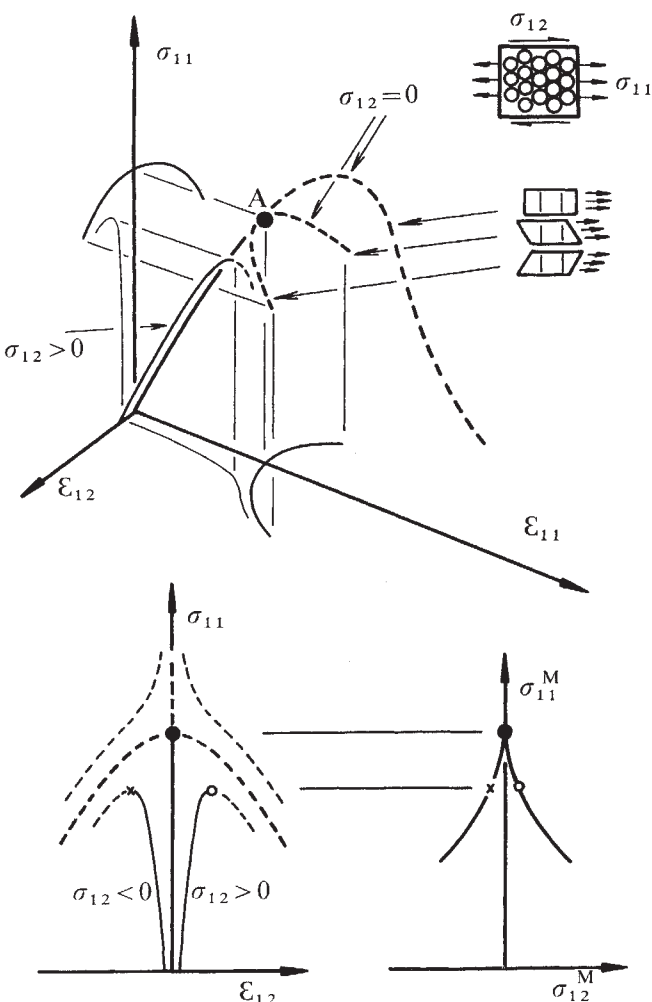


Fig. 2 This shows how Fig. 1 is modified by the addition of the direct stress $\sigma_{22} = \sigma_{22}^C$ which has shifted the point of bifurcation to the maximum of the primary stress-strain curve. The failure locus is now locally bilinear.



law cusp in the failure-stress locus represented by the plot of the peak stresses σ_{11}^M and σ_{12}^M . This locus corresponds to the well known imperfection-sensitivity curves in the buckling of engineering structures⁶⁻⁸.

We have now extended this study by adding additionally a second direct tensile stress σ_{22} on the horizontal faces of the lattice. Within our approximations², which include an assumption that the vertical close packed columns undergo a negligible change in length, this direct stress has no effect on the primary equilibrium path lying in the plane of σ_{11} and ϵ_{11} . It does, however, influence the stability of this path, and has the effect of displacing the critical branching point A along the path. As we would expect, a negative (compressive) stress displaces A towards the unloaded state while a positive (tensile) stress has a stabilising action and displaces A in the direction of positive ϵ_{11} .

We have evaluated the magnitude of this stress, σ_{22}^C , that is necessary to shift the point A precisely to the maximum of the primary equilibrium path to give the situation shown in Fig. 2. In the presence of this small tensile stress σ_{22}^C the crystal loaded by σ_{11} will thus encounter a hill-top branching point² at which the two-thirds power law cusp is replaced by a bilinear failure locus as shown in the plot of the peak stresses σ_{11}^M and σ_{12}^M .

Inspecting our equations and the form of the equilibrium paths it is easy to see that this hill-top branching point is in fact

Thom's hyperbolic umbilic catastrophe³⁻⁵, so that the full three dimensional failure locus in the space of the planar stresses σ_{11}^M , σ_{12}^M and σ_{22}^M must have the form of Fig. 3. Here we see that on the crest defined by $\sigma_{12}^M = 0$ the sharp cusp degenerates at the singularity corresponding to $\sigma_{22}^M = \sigma_{22}^C$ into two straight lines and thereafter the curve has a parabolic form. This crest is curved between A and C where it corresponds to failure at a branching point, but it is straight and parallel to the σ_{22} axis between C and B where failure is associated simply with the maximum of the primary equilibrium path: in this latter range the bifurcation has moved beyond this maximum and therefore no longer initiates the failure of the crystal. The failure surface has the characteristic form of the stability boundary of the hyperbolic umbilic catastrophe⁹ which has often been likened to a breaking wave.

This attractive and concrete example of the hyperbolic umbilic catastrophe can now be added to the imperfection-

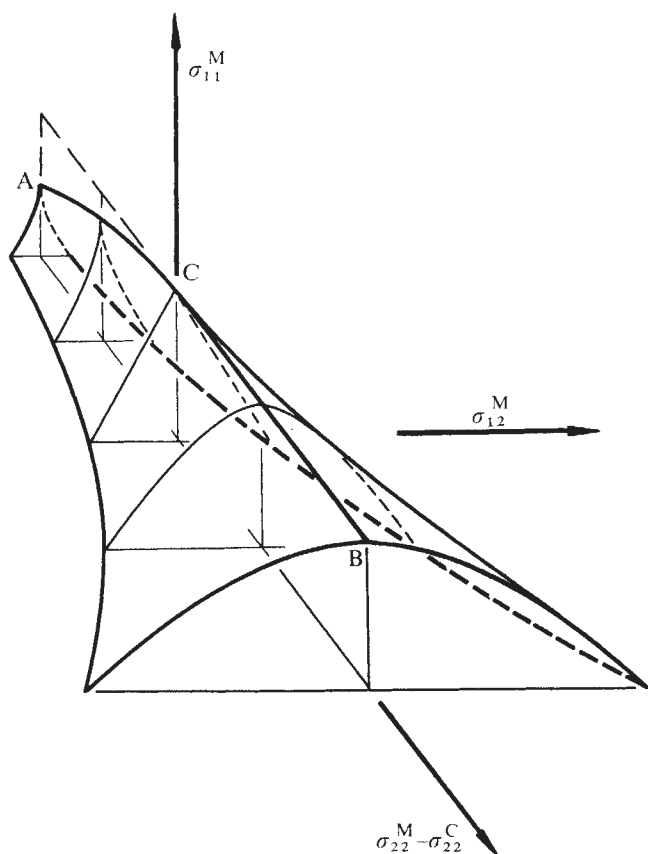


Fig. 3 The stability boundary in the space of the failure stresses corresponding to the catastrophe boundary of the hyperbolic umbilic catastrophe.

sensitivity curve determined by G. W. Hunt^{1,6} for the buckling of a stiffened panel as a second illustration of this catastrophe in nonlinear elasticity¹⁰.

We feel that our demonstration of this new instability phenomenon for a fairly idealised, perfect and dislocation-free lattice is of some fundamental importance and is a useful complement to the numerical studies of Macmillan and Kelly¹¹ and the theoretical work of Hill¹². Some further details of our cusp analysis have been published¹³, and details of the present extension will be presented shortly.

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Received February 9; accepted March 8, 1976.

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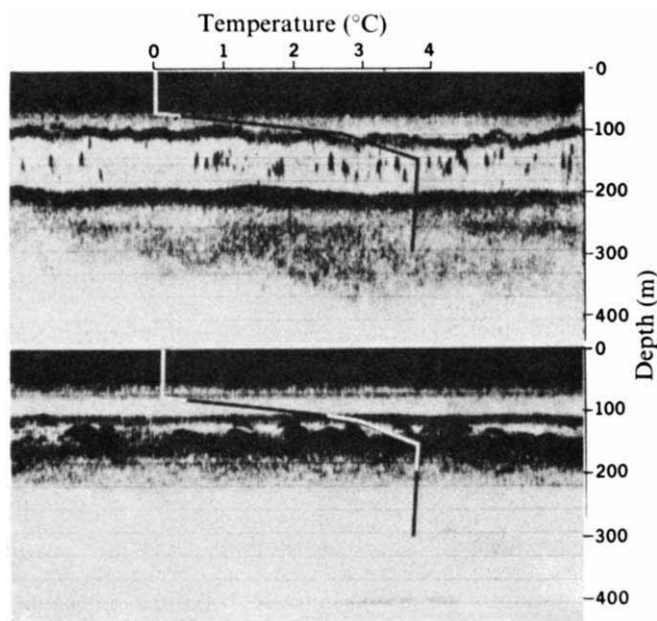
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Arrest of deep scattering layer migration by a positive thermal gradient

LIGHT and temperature are considered to be the factors that influence diel vertical migrations of populations of biological sound scatterers (deep scattering layers) in the sea¹⁻³. While light is probably the most important, temperatures which surpass the physiological tolerances of the organisms should play a major role in setting vertical limits. With positive thermal gradients (temperature increasing with depth) as are found in arctic and sub-arctic seas, sufficiently low temperatures near the surface could limit the evening ascent. This situation was observed in March, 1966 over the Kurile Trench along the eastern coast of the Kamchatka Peninsula (50°30'N, 162°30'E), an area influenced by the Oya Shio current system. Data were collected from USNS Charles Davis; acoustic records were made on a Westrex MK 10A Precision Depth Recorder using a Giffit transceiver to drive a hull-mounted UQN transducer at 12 kHz. The records were made on the 1.5-s sweep scale of the recorder.

Day and night acoustic scattering profiles are shown in Fig. 1. The day record was made between 1430 and 1530, March 5 while the ship was underway at 10 knots. The night record was made between 2330, March 5 and 0030, March 6 while the ship was drifting. Both records were made on the same station, however, and within a distance of ten nautical miles. The sea floor was at ~6,500 m. A 1,000-m hydrographic cast was taken at 2000, March 5 and

Fig. 1 Acoustic scattering profiles recorded over the Kurile Trench in March 1966. The upper record was made between 1430 and 1530 with the ship underway at 10 knots; the lower between 2330 and 0030 with the ship drifting. Temperature profile data from a hydrographic cast is superimposed.



the temperature structure is superimposed. Seven expendable bathythermographs taken within a ten-mile radius of the station show a similar profile. Salinity was ~ 33.2 ‰ from the surface to 100 m; at 200 m it had increased slightly to > 34 ‰.

The first well defined scattering feature below the outgoing pulse and surface scattering was a 20-m wide layer which oscillated between 90 and 130 m, the depth of the thermocline. The layer did not migrate, but seemed permanently associated with the thermocline on both day and night records. This phenomenon has been previously reported, and it has been demonstrated that the density gradient of the thermocline could not itself be the scattering factor for frequencies as high as 12 kHz (refs 5 and 6). Oscillations of the layer are suggestive of internal waves on the thermocline.

Between the thermocline layer and the main scattering layer were many discrete reflectors. Little is known of the composition of these targets over the deep sea, but their recordings are similar to acoustic signatures of fish schools in coastal waters⁶. The spatial relationship between these targets and the layers was maintained on both the day and night recordings. Like the main scattering layer beneath them, their upward migration was apparently arrested by the low temperature.

Maximum depth to the top of the main scattering layer was 255 m at 1000, March 5. Below the dense upper component of the layer, diffuse scattering was present to 350 m. Upward migration began at 1300 and a very gradual ascent (~ 20 m h⁻¹) progressed through the afternoon. The ascent was terminated at 1800 at a depth of 145 m.

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A- and B-type hydrogen bond modifications of potassium hydrogen tartronate

ACCORDING to Speakman¹ two types of intermolecular hydrogen bonds between carboxyl groups can be discerned in the crystal structures of acid salts of carboxylic acids. The first, type *B*, occurs when two carboxyl groups are not symmetry related, and have structural differences sufficient to characterise them, separately, as unionised ($-\text{CO}_2\text{H}$) and ionised ($-\text{CO}_2^-$). Thus, any hydrogen bond between these groups must be asymmetric. The second, type *A*, occurs when two carboxyl groups are related by a symmetry element of the crystal, so that they are effectively equivalent. Thus any hydrogen bond between the groups must be, at least formally, symmetrical.

A reliable tool for establishing the type of hydrogen bond present in crystals of acid salts is infrared spectroscopy, which can be used to examine the region of the stretch vibration of the hydroxyl group². Also, geometric evidence from X-ray data on a number of acid salts unambiguously reveals the existence of the two types of hydrogen bonds. The difference in symmetry brings about a significant difference in the geometry of the carboxyl groups involved in hydrogen bonding¹.

Moreover, there is also a difference in the $\text{O}\cdots\text{O}$ distance: in the *A* type it seems to be at least 0.05 Å shorter than the *B* type. It has been argued that the symmetry in the *A* type could be only apparent, and attributable to a 1:1 disordering of the

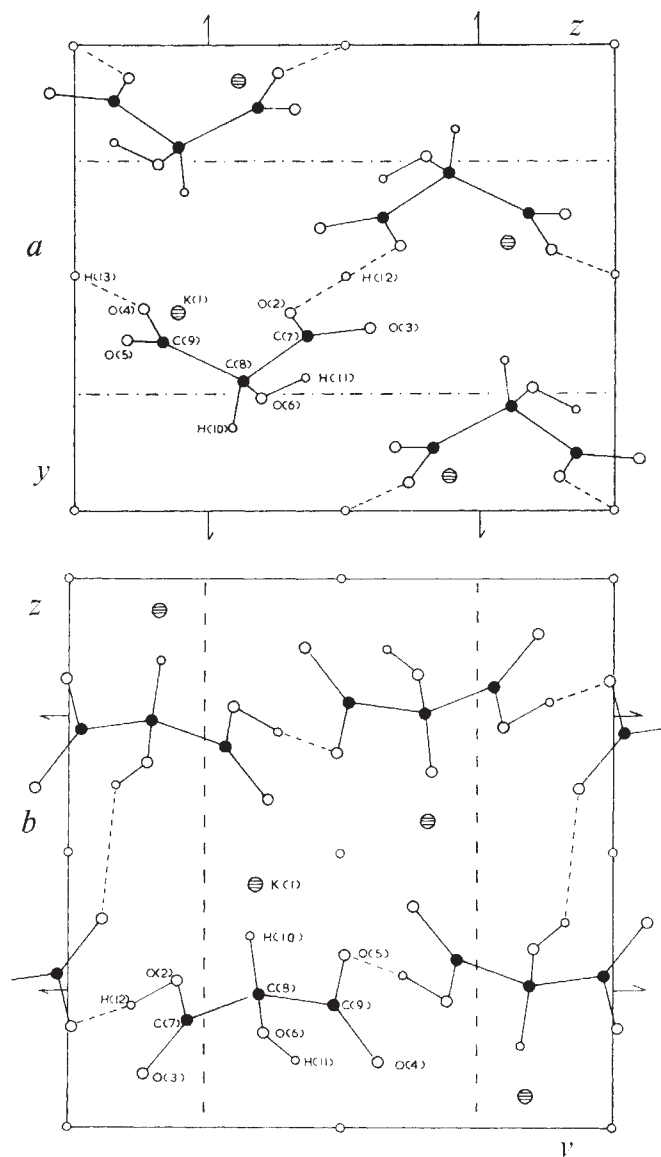


Fig. 1 Projections along the *a* axis of the crystal structures of potassium hydrogen tartronate: *a*, α form; *b*, β form. Dashed lines, hydrogen bonds.

hydrogen atom in an asymmetric hydrogen bond³. But it seems from infrared and X-ray studies that *A*- and *B*-type hydrogen bonds do differ. It is intriguing to identify the factors that determine whether a compound crystallises either as an *A*- or *B*-type salt. It is of interest to note that in the crystal of potassium hydrogen *meso*-tartrate both types of bond are present^{4,5}. The *A* type relates to one *meso*-tartrate carboxyl group, and the *B* type relates to the second, which is inequivalent to the first because of molecular asymmetry.

The study of the *A*- and *B*-type hydrogen bonds found in the acid potassium salt of tartronic acid seems to be more promising in studies of the problem. That compound crystallises in two forms, the α form having an *A*-type and the β form having a *B*-type hydrogen bond. Experimental data of the modifications are summarised in Table 1, and relevant structural data are given in Table 2.

The *A*- and *B*-type hydrogen bonds are easily recognised from projections along the *a* axis (Fig. 1). The tartronic acid anions in both structures are linked by hydrogen bonds forming infinite chains. In the α form, the chains are generated by centres of inversion lying along the $[101]$ direction with $\text{O}\cdots\text{O}$

Table 1 X-ray data of the crystal structures of potassium hydrogen tartrate

From ethanol-water solution α form	From aqueous solution β form
Monoclinic, $P2_1/n$ $a = 7.356$; $b = 8.116$; $c = 9.197$ (Å) $\beta = 94.81^\circ$, $V = 547.1$ Å ³ $D_x = 1.92$ g cm ⁻³ ; $Z = 4$ 1,129 independent reflections	Monoclinic, $P2_1/c$ $a = 6.532$; $b = 9.248$; $c = 9.505$ (Å) $\beta = 99.74^\circ$, $V = 565.8$ Å ³ $D_x = 1.86$ g cm ⁻³ ; $Z = 4$ 1,204 independent reflections
MoK α radiation, $\lambda = 0.7107$ Å	
Absorption correction	
μ (MoK α) = 9.7 cm ⁻¹	μ (MoK α) = 9.4 cm ⁻¹
Block-diagonal least-squares refinement K, O, C: positional and anisotropic thermal parameters. H: positional parameters, B fixed; extinction coefficient $c = (e^2/mc^2)^2 \lambda^2 / V^2 r^*$	
$\epsilon = 29.10^{-5}$ cm ⁻¹ $R = 0.042$	$\epsilon = 34.10^{-4}$ cm ⁻¹ $R = 0.042$

distances of 2.433 and 2.435 Å, respectively. In the β form the O...O distance in the hydrogen bond propagating along the screw axis is 2.485 Å. Glide planes interrelate the anion chains in both types. The aliphatic hydroxyl group interaction is very different in the two forms. In view of its geometry (Table 2), it is highly questionable whether the O-H...O contact in the α form should be considered as a hydrogen bond. In this respect we refer to the crystal structure of tartaric acid itself⁶, in which the aliphatic hydroxyl group is free⁷: (O...O, 3.02 Å; H...O, 2.24 Å; O-H...O angle, 142°).

In contrast, the OH group in the β form participates in a hydrogen bond of normal geometry (Table 2). The molecular geometry and conformation in the anion part of the α form differs significantly from that of the β form. In the former the conformation of one of the carboxyl groups with respect to the C(α)-hydroxyl group is antiplanar, which is quite exceptional⁸. The observed geometry of the carboxyl group reflects the schematical classification in *A* and *B* types: the *A*-type carboxyl groups are half ionised, whereas in the *B*-type anion one carboxyl group is neutral and the other is completely ionic (Table 2). The potassium coordination is eightfold and, as usual, irregular.

From the shorter O...O distances in *A*-type hydrogen bonds it looks as though such a symmetrical bond is more favourable than an asymmetric one. We feel that the comparison of the crystal structures of the two modifications of potassium hydrogen tartrate provides new evidence for this. The energetically

unfavourable molecular conformation and the weaker O(6)-H(11)...O(5') hydrogen bond may compensate for the stronger *A*-type hydrogen bonding in the α form, and thus account for the dimorphism.

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Speed of podzolisation

THE coastal sand dunes of eastern Australia are being mined extensively for heavy minerals. After mining, the replaced sand is subject to soil-forming processes. On such sites we have observed the formation of podzol profiles after 4.5 yr, with the development of bleached A₂ 'pipes' up to 45 cm deep after 9 yr, indicating the extreme rapidity of soil-forming processes in suitable conditions.

Only a few estimates have been made of the time required for a podzol profile to develop because the time of initiation of soil-forming processes cannot usually be determined precisely. Chandler¹ used the known retreat pattern of the Medenhall glacier in Alaska and by comparing the degree of podzol development in materials of different ages, estimated that a mature profile would require 1,000–1,500 yr to form. Tamm (quoted by Russell²) used the drainage of Lake Ragunda in 1796 as the beginning of soil development and also estimated a time of from 1,000–1,500 yr for the development of a mature podzol. In Australia, Burges and Drovers³ examined soil development on a sequence of beach ridges just north of Sydney, and estimated that a period of 1,000 yr is required to develop a distinct B horizon in an iron podzol. The basis of this estimate was not the soil profile itself, but the supposed age of the beach ridges in which the podzols are developed.

On a more restricted time scale, Crompton⁴ demonstrated that thin iron pan podzols, not more than 8 cm in total thickness have developed in less than a century. Muir⁵

Table 2 Some geometric details of the carboxyl groups and hydrogen bonds in the α and β forms of potassium hydrogen tartrate*

	α form	Δ	β form	Δ
C(7)-O(2)	1.277(3)		1.302(4)	
C(7)-O(3)	1.223(3)	0.054	1.218(4)	0.084
C(9)-O(4)	1.281(3)		1.233(4)	
C(9)-O(5)	1.226(3)	0.055	1.277(4)	0.044
C(8)-C(7)-O(2)	114.6(2)		112.1(2)	
C(8)-C(7)-O(3)	119.3(2)	4.7	123.8(3)	11.7
C(8)-C(9)-O(4)	115.0(3)		118.7(3)	
C(8)-C(9)-O(5)	120.5(2)	5.5	116.1(2)	2.6
Hydrogen bond DHA	D...A	D...H	H...A	D...H...A
(α form)				
O(2)H(12)O(2')	2.433(4)	1.216(2)	1.216(2)	180
O(4)H(13)O(4')	2.435(4)	1.218(2)	1.218(2)	180
O(6)H(11)O(5')	2.885(4)	0.85 (5)	2.42 (5)	114(3)
(β form)				
O(2)H(12)O(5')	2.485(3)	1.06(5)	1.43(5)	173(3)
O(6)H(11)O(4')	2.860(3)	0.92(3)	2.24(5)	152(3)

*Distances in Å; angles in degrees; e.s.d. in parentheses.

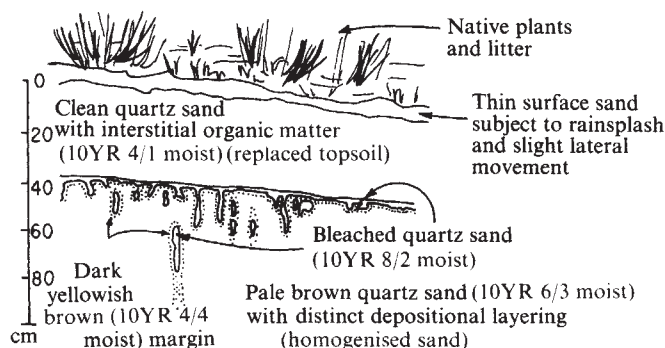


Fig. 1 Soil profile in revegetated sand 9 yr after mining (sketch from photograph).

reported that on formerly cultivated land overgrown with Scots pine in Scotland a bleached A_2 , 0.5 cm thick, developed in 20 yr.

The example we have studied is in the Myall Lakes area, 290 km north of Sydney (Portion 19, Parish of Fens, New South Wales, Private Mining Lease 2, 32°39'S 150°11'E). Here, before mining, well developed podzols supporting dry heath or woodland occurred in low relief quartz sand dunes. The heath vegetation was similar to that described by Clark⁶ and included shrubs such as *Banksia*, *Leptospermum*, *Casuarina* and *Persoonia*, up to 3 m high. The original profile was: dark grey A_1 0–30 cm; bleached A_2 30–150 cm, sharply demarcated across a highly convoluted boundary from the B_2 which had a marked colour contrast but only slight and sporadic induration developed in several metres of sand.

The oldest site examined supported heath and was mined in September 1966. During this process the podzol was completely destroyed, since mining involves the stripping and stockpiling of the topsoil (largely A_1), and complete homogenisation of all remaining sand to depths greater than 5 m by the dredging and mineral separation processes. The pale brown (10 YR 6/3 moist 7/3 dry) homogenised sand was reformed to a similar topography as the original and covered by the stockpiled topsoil during March 1967. The area was planted with rye (*Secale cereale*), West Australian blue lupin (*Lupinus angustifolius*) and fertilised with Shirleys No. 17 fertiliser (9.2% N, 4.3% P, and 4.6% K) at the rate of 517 kg ha⁻¹, during September 1967. Aerial fertilisation with Nitram (34% N) at the rate of 129 kg ha⁻¹ was carried out in April and September 1972. Regrowth of native species occurred from seed in the replaced topsoil. The present vegetation on the mined site is 0.5–1.5 m high, covers 50% of the ground surface (visual estimate) and is made up of shrubs which occur on adjacent unmined areas but in different proportions.

Figure 1 is a section through the sand 9 yr after mining. Below the abrupt lower boundary of the replaced topsoil, the underlying homogenised sand has a zone of bleaching with an extremely convolute lower margin marked by a thin zone of dark yellowish brown (10 YR 4/4 moist and dry) sand. On examination under a binocular microscope the surface organic-rich material (replaced A_1) is seen to consist of clean quartz sand grains with interstitial organic matter. The homogenised unbleached sand is made up of approximately 80% clean quartz grains and 20% of grains with reddish (probably ferric oxide) skins on their surface, giving the overall pale brown colour. Within the bleached zone the surfaces of the quartz grains are completely clean and there is no interstitial organic matter. Bleaching of the pale brown homogenised sand is associated with a reduction of the iron content of the sand from about 1,150 p.p.m. (w/w) to about 250 p.p.m. Iron was extracted from ashed samples in hot concentrated hydrochloric acid

and concentrations were determined by atomic absorption spectrophotometry. Dissection of Kubierna tin samples shows the bleached zone to be planar immediately beneath the replaced topsoil, to average 1–2 cm in thickness and to extend up to 45 cm deep as scattered roughly circular pipes. On a microscale this is an exact replica of the convoluted relationship seen between the A_2 and B_2 horizons of the podzols in unmined sand. It would seem to result from percolating solutions removing surface coverings of ferric oxides from quartz sand grains and depositing them again within a short distance, that is, in a typical podzolising process. The rapidity of this process in producing features of the dimensions shown in Fig. 1, in 9 yr is probably due to the absence of finer grained materials to inhibit the throughflow of solutions or to inactivate the molecules responsible for the podzolisation. It is not certain whether the active organic molecules originate from the present vegetation or from organic matter in the replaced topsoil. The pipe-like areas of bleached sand were also found on mined sand under *Imperata cylindrica* and in an area of sparse vegetation on a former *Ammophila arenaria* nursery. They were not, however, present under this vegetation where the organic content of the replaced topsoil was low.

The speed with which the pale brown quartz sand can be bleached has been demonstrated in the laboratory. Aqueous extracts from fresh leaves of *Angophora costata* and *Eucalyptus gummiifera* growing on a podzol site were prepared using the method of Bloomfield⁷. These species are common in woodland on podzols at Myall Lakes. The 5% extract was dripped at 1–2 cm³ h⁻¹ into the top of a glass tube (0.3 cm internal diameter) packed with the homogenised mined sand. Continuous flow produced visible bleaching to a depth of at least 1 cm in the first hour. This extended to the bottom of the column (25 cm) after about 7 h. With interrupted flow but without drying out of the column, a bleached layer 2 cm deep formed above a dark brown band 0.1 cm thick within 18 h. Water or acetate buffer (pH 4) did not duplicate these effects. These experiments reproduce within hours aspects of the podzol morphology. They are being continued in an effort to determine the conditions for iron redeposition and the relative importance of vegetation species and soil organic matter in the genesis of these profiles.

Two points requiring evaluation arise from these observations; pedological and geomorphic ages based on the degree of development of podzol profiles, especially in sand dune areas, may have been overestimated and the speed of pedological processes, especially in disturbed systems, may have been underestimated.

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Transfer of mildew resistance from the wild oat *Avena barbata* into the cultivated oat

MILDEW (*Erysiphe graminis*, f.sp. *avenae*) can reduce the yield of cultivated oats by 30% (ref. 1) and is the most important disease of the crop in the UK. The ability of the pathogen to change either by mutation or recombination, giving rise to strains which can effectively attack previously resistant cultivars, leads to a continuous search for further sources of resistance. Genotypes of related weed species of *Avena* have been isolated that are completely resistant to mildew. A genotype of the tetraploid species *Avena barbata* ($2n = 28$) collected in Algeria is resistant to all the prevalent races of mildew and would form a valuable source of resistance to the pathogen. We now report the successful transfer of this source of mildew resistance into the cultivated oat. This provides the oat breeder with a new source of mildew resistance to use in breeding programmes.

The cultivated oat *Avena sativa* is a hexaploid species ($2n = 42$) and probably combines three distinct diploid sets of chromosomes. *A. barbata* can be crossed readily with the cultivated oat, but the hybrid is sterile. It is possible to obtain the occasional viable seed when the sterile hybrid is backcrossed to *A. sativa* and some success has been achieved in transferring genes for crown rust resistance from diploid and tetraploid weed species to *A. sativa* by continuous backcrossing^{2,3}. But the genetic control of chromosome pairing in *A. sativa*^{4,5}, which restricts pairing to homologous chromosomes, minimises the amount of interspecific chromosome pairing that takes place in hybrids and derivatives. Consequently, plants resistant to crown rust isolated in later generations of interspecific hybrids have often proved to be chromosome substitution or addition lines, and not the products of natural recombinations⁶⁻⁸.

The amphiploid *A. barbata* × *A. sativa* was produced at the Welsh Plant Breeding Station and backcrossed twice to *A. sativa*. Selection for mildew resistance was practised at each generation and a resistant plant with 44 chromosomes was isolated from the selfed progeny of the second backcross generation⁹. This disomic addition line combines the full complement of the recurrent *A. sativa* parent Manod and a pair of *A. barbata* chromosomes. The monosomic addition line ($2n = 43$) is also resistant. When the selfed progenies of the monosomic addition line were tested for mildew reaction to race 2 all the resistant plants were found to contain the *A. barbata* chromosome, confirming that resistance is due to the presence of the *A. barbata* chromosome. No recombinants were found in the progeny (5,649 seedlings tested) of the monosomic addition line.

Seeds of the disomic addition line ($2n = 44$) were irradiated at doses of 10,000 and 15,000 rad using a ⁶⁰Co source. The screening procedure for translocations was that used successfully in wheat-rye studies¹⁰. If translocations between the *A. barbata* and *A. sativa* chromosomes were achieved, the formation of a multivalent or incompletely homologous bivalents would give rise to gametes deficient for the *A. barbata* gene for resistance. When the I_1 plants (originating from the irradiated seeds) are selfed fusion of gametes which do not have the resistant gene should result in some susceptible progeny in the I_2 populations. Nineteen I_2 families out of 391 did segregate a proportion of susceptible progeny but the rest produced only resistant seedlings, as expected from a normal disomic addition line. The resistant plants from the 19 I_2 families were grown to maturity to produce I_3 seeds. As a result of further tests for mildew reaction in the I_3 and I_4 generations, lines were isolated which segregated three resistant: one susceptible seedlings. All these segregating lines had 44 chromosomes, which shows that the susceptible seedlings were not the result of the complete loss of the *A. barbata* chromosome.

One of these particular lines, B.133, was studied in detail to assess the potential of the translocation stock as breeding

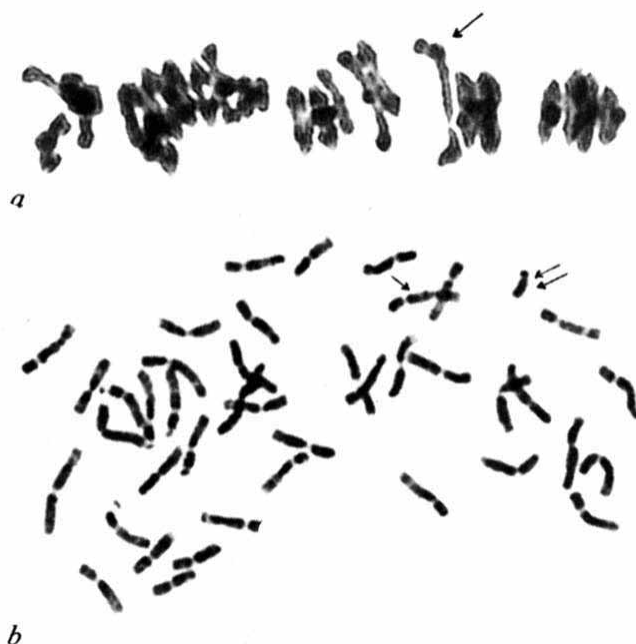


Fig. 1 a, Metaphase I in I_3 plant of B.133 with 22 bivalents, one of which is a distinct heteromorphic bivalent. b, Somatic chromosomes of a euploid plant heterozygous for the translocated chromosome. Two arrows indicate natural chromosome 21 and a single arrow the translocated chromosome.

material. Meiotic analysis was made difficult by clumping and stickiness of chromosomes, but in some cells a distinct heteromorphic bivalent (Fig. 1a) was observed. Resistant plants were crossed with the susceptible cultivar Manod to produce 43-chromosome hybrids. The F_2 families again segregated in a 3 : 1 ratio, the pooled data being 361 resistant : 120 susceptible seedlings. Counting the chromosomes of the resistant seedlings revealed that with the exception of the occasional seedling with 43 chromosomes the resistant progeny had the euploid number of 42 chromosomes. The *A. barbata* gene had been incorporated into the *A. sativa* genome and behaved normally, producing a monofactorial ratio.

Some of the chromosomes of *A. sativa* can be identified readily according to shape and size¹¹. Chromosome 21, the smallest of the complement, can be identified easily. In the F_2 families which segregate three resistant : one susceptible we found that in the resistant seedlings chromosome 21 was either absent or only present as a single chromosome. In the susceptible seedlings the pair of chromosomes 21 was always present. Because all the seedlings had the euploid number of 42 chromosomes the fact that chromosome 21 was either absent or present single in the resistant seedlings shows that it is involved in the translocation. Resistant seedlings lacking chromosome 21 are homozygous for the translocated chromosome and those containing only a single chromosome 21 are heterozygous for the translocated chromosome. It has already been established that the gene for mildew resistance is located on the short arm of the *A. barbata* chromosome⁹. The morphology of the translocated chromosome (Fig. 1b) suggests that it involves the short arm, the centromere and a portion of the long arm of the *A. barbata* chromosome and the long arm of chromosome 21 of *A. sativa*.

Plants homozygous for the translocation are vigorous and fertile, the deletion of a part of chromosome 21 and the duplication arising from the *A. barbata* segment are readily tolerated. In the euploid lines which were heterozygous for the translocation 21 bivalents were regularly formed, one of which was heteromorphic. The regular monofactorial segregation observed when the heterozygous translocation stock is selfed shows that the translocated chromosome can compete effectively with the intact chromosome 21. It should thus be possible to

transfer the mildew resistance to other cultivars of the cultivated oat by a simple backcrossing programme.

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Received January 19; accepted February 25, 1976.

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Cyanogenic polymorphism in bracken in relation to herbivore predation

THE ecological success of bracken is partly a result of its extensive rhizome system, and because of its ability to synthesise various secondary compounds which deter predators and phytopathogens¹⁻³. These compounds include the cyanogenic glycoside, prunasin⁴, toxic because on enzymatic hydrolysis HCN is released. Although present in only 5% of ferns⁵, cyanogenic glycosides are ecologically significant in other plant species; they deter both molluscs and voles from grazing on *Lotus corniculatus*⁶, and rabbits from eating *Trifolium repens*⁷. As in these two species, we have found that cyanogenesis is also polymorphic in bracken.

Our aim was to correlate herbivore predation with changes in cyanogenesis and phenolic content of bracken throughout the growing season (G.A.C.-D., E. Bernays, S. Finch and T.S., unpublished). We examined bracken from several randomly chosen sites in Richmond Park, Surrey. These comprised open and shady areas, and involved both inter- and intra-population studies. Our results showed that in most populations 96% of individuals contained both prunasin and the corresponding hydrolase and were therefore cyanogenic, but in a few populations, 98% of individuals were acyanogenic. Of these, 85% lacked both enzyme and substrate, whereas 15% contained prunasin without enzyme. We found that the bracken fronds from such areas were heavily grazed by deer and sheep, as examination of animal droppings in the area and observations by the gamekeeper showed. Examination of other widely separated patches of bracken confirmed that only fronds from acyanogenic plants were eaten.

To establish whether it is the production of HCN or merely the possession of prunasin which is the predator deterrent, we fed disks of bracken fronds to the phytophagous locust, *Schistocerca gregaria*. The results of our experiments showed that when both prunasin and enzyme were present only 4% of fronds were eaten, whereas when only the substrate was present the insects consumed 52% of the test meal, which was nearly the same as when both substrate and enzyme were absent (48%). It seems, therefore, that the production of HCN (or benzaldehyde) acts as the deterrent. Young fronds of bracken were more cyanogenic than older fronds, whether sampled throughout the season, or as young and spore-bearing fronds of a single plant. We found that *S. gregaria* consumed the minimum amount of bracken in test meals during late May to early June, coinciding with the greatest intensity of cyanogenesis⁸.

We conclude that cyanogenic polymorphism in bracken has a positive role in determining the degree of herbivore predation and that there is selectivity for acyanogenic forms in natural conditions.

We thank Dr E. Bernays, C.O.P.R., Mr S. Finch, Brunel

University, and Mr Brown, gamekeeper of Richmond Park, for their assistance. This work was supported by the ARC.

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Received January 21; accepted February 26, 1976.

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Inhibitory binocular interaction in human vision and a possible mechanism subserving stereoscopic fusion

IN the human visual system, pathways from the two eyes converge anatomically by partial crossing over of the optic nerve fibres at the optic chiasma. Neurophysiological studies of the primary visual cortex of primates have established that these converging pathways carry signals from both eyes to the same cortical neurones¹. In normal viewing conditions, each eye forms an image of the external visual field, the point of attention in the field being centrally fixated in both eyes. There is a small difference in the relative positions of non-central image points formed on the two retinas because the eyes view the visual field from slightly different positions. The ability of the primate visual system to extract information about the depth of a focused object in the visual field apparently rests on the convergence on to single cortical neurones of signals arising from the slightly displaced images in the two eyes². If each eye perceives a totally different visual stimulus, however, the visual signals arising from the two stimuli are perceptually antagonistic and, in this situation, an observer usually detects only one stimulus at a time. This phenomenon is referred to as binocular rivalry. We have investigated interactions between visual signals produced by two different stimuli when presented one to each eye.

Our studies concerned a special class of stimulus patterns—one-dimensionally periodic distributions of light which, because of their alternating light and dark bar structure, are called grating stimuli (Fig. 1 inset). The visual detectability of such grating stimuli is frequently specified by the minimum (or threshold) value of contrast level at which the grating can just be detected, with the contrast defined as $(I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$, $I_{\max}$ and $I_{\min}$ being respectively the maximum and minimum illumination levels present in the grating stimulus pattern. It has been shown that the threshold contrast level for detection of a grating increases transiently after adaptation to a similar grating of high contrast³⁻⁵. This adaptation effect is observed only when the test and adaptation gratings are of similar spatial frequency (that is periodicity) and when they are oriented in near parallel directions. The adaptation effect can be studied by presenting both test and adaptation gratings to the same eye⁶, or by presenting them one to each eye⁶, and both methods yield essentially the same results. We measured the grating adaptation effect with the test and adaptation gratings, matched in orientation and spatial frequency, presented to one eye and a further grating stimulus, which we call the conditioning grating, presented to the other eye (Fig. 1 inset). The results show that when the conditioning grating is of spatial frequency, f_{cg} , in the approximate range 1-4 cycles per degree, it suppresses

the adaptation for all spatial frequencies, f_{ag} , of the test and adaptation gratings, except when f_{ag} is equal to f_{cg} . With the observer's head in the normal upright position, this suppression effect is observed only when all the gratings are orientated in a vertical direction and only when the conditioning and adaptation gratings are presented to different eyes.

The experimental arrangement of the visual fields is illustrated in Fig. 1 (inset). The grating patterns were produced by laser interference methods^{6,7} and were in all cases sinusoidally modulated grating patterns of 100% contrast. The adaptation effect was measured in terms of Δ , equal to $\log I - \log I^1$, where I is the mean threshold illumination level of the 100% modulated test grating required for its detection after adaptation to the adaptation grating and I^1 the corresponding illumination level following adaptation to a uniform field. The illumination level of the uniform field and the mean illumination level of the adaptation grating were set equal at 4.5 log trolands, thus ensuring that both stimuli produced the same mean retinal adaptation condition. At the start of each measurement, the observer viewed the adaptation grating for 4 min and was instructed to scan the field during the periods of adaptation to ensure uniform retinal adaptation. As the grating adaptation effect is of short duration⁸, the test and adaptation gratings were then alternated for periods of 3 and 20 s, respectively, the cycle being repeated until the observer obtained the threshold illumination level, I , for detection of the

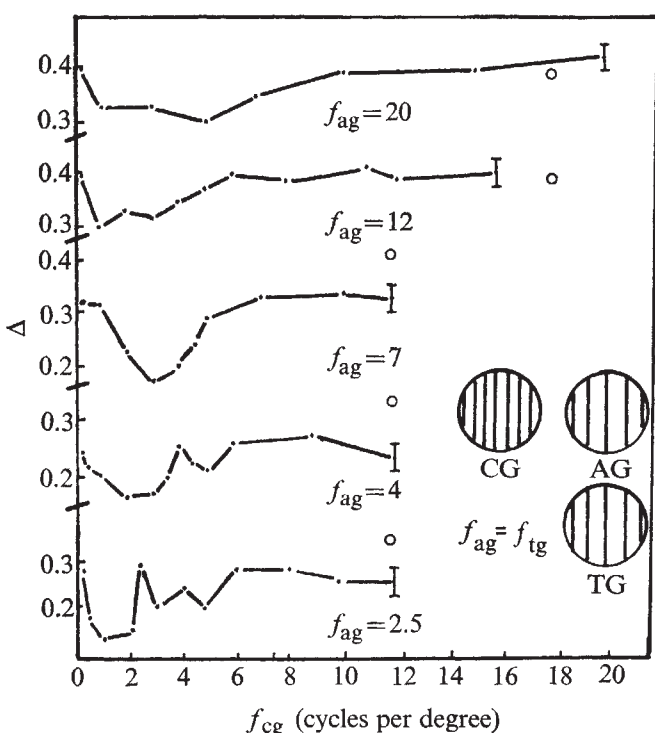


Fig. 1 Values of Δ plotted against the spatial frequency, f_{cg} , in cycles per degree of the conditioning grating. Plots are given for five spatial frequencies, f_{ag} , of the test and adaptation gratings and the values of f_{ag} corresponding to each plot are given in cycles per degree to the right of the data points. Experimental points, denoted by the full circles, are the mean of 10 observations, and typical standard errors are denoted by the vertical error bar given with the highest frequency point of each plot. All other standard error values were similar in amplitude to those defined by the bars. For each value of f_{ag} , Δ was also determined in the absence of the conditioning grating, and the values obtained in this way are plotted as open circles to the right of each plot. All grating stimuli were of wavelength 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands throughout. All gratings were orientated in a vertical direction. The inset shows the appearance of the visual fields in the two eyes. During periods of adaptation, the conditioning grating, CG, was presented to the left eye and the adaptation grating, AG, to the right eye. The test grating, TG, was presented in isolation to the right eye. All gratings fields were 30°.

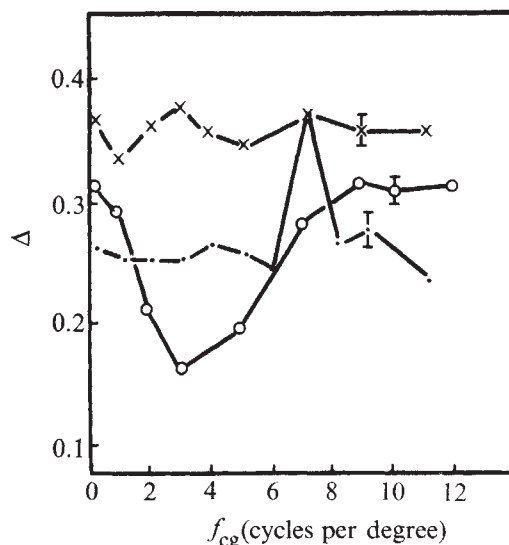


Fig. 2 Values of Δ plotted against the spatial frequency of the conditioning grating, f_{cg} , in cycles per degree. All values refer to test and adaptation gratings of spatial frequency 7.0 cycles per degree. Open circles refer to the stimulus configuration shown in Fig. 1, that is, with all gratings orientated vertically, the adaptation and conditioning gratings presented simultaneously to the right and left eyes, respectively, and the test grating presented to the right eye. Crosses refer to a similar stimulus configuration, but with all gratings orientated in a horizontal direction. Full circles refer to the arrangement in which the adaptation and conditioning gratings were both presented to the same eye as the test grating. All data points are the mean of 10 observations and typical standard error values are denoted by the vertical error bars. All stimuli were of wavelength 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands. $f_{ag} = f_{tg} = 7$ cycles per degree.

test grating. A similar procedure, in which the adaptation grating was replaced by a uniform field of the same mean illumination level, was used to determine I^1 . The conditioning grating was presented in coincidence with the adaptation grating (Fig. 1 inset) and the observer's head was fixed by means of a dental clamp with the interocular direction horizontal. Data were obtained for three observers, all of whom gave substantially similar results, and the data presented here refer to E.W. who made the most extensive observations.

The results obtained with the grating bars orientated in a vertical direction are given in Fig. 1, values of Δ being plotted against f_{cg} , the spatial frequency of the conditioning grating. Data are given for a number of values of f_{ag} , the common spatial frequency of the test and adaptation gratings, and the values of Δ obtained in the absence of the conditioning field are also given for each value of f_{ag} . These data show that there is a small decrease in Δ , relative to the value obtained in the absence of the conditioning stimulus, over much of the range of f_{cg} . For values of f_{cg} in the approximate range 1–4 cycles per degree, however, there is a large decrease in Δ , implying that the adaptation effect is suppressed in the presence of the conditioning grating. An exception to this is observed when the conditioning and adaptation gratings have the same spatial frequency, for example, at f_{cg} equal to 2.5 cycles per degree for the data referring to f_{ag} equal to 2.5 cycles per degree. When the experiment was repeated with the gratings orientated horizontally, however, the data showed that for all values of f_{cg} , except f_{cg} equal to f_{ag} , Δ is reduced by about 0.1 relative to its value in the absence of the conditioning grating (full circles in Fig. 2). Measurements with the gratings set at various angles between vertical and horizontal established that the grating adaptation effect is suppressed only for vertical and near-vertical orientations of the grating lines (Fig. 3). Further experiments were performed in which the adaptation and conditioning grating were both presented to the same eye as the test grating, all three gratings being orientated vertically. A

typical set of data is given in Fig. 2 (crosses), values of Δ being plotted against f_{cg} , the spatial frequency of the conditioning stimuli. The large reduction in Δ for f_{cg} in the range 1–4 cycles per degree found when the adaptation and conditioning gratings are presented to different eyes, does not occur when they are presented to the same eye. Thus the suppression of the grating adaptation effect by a low frequency conditioning grating is specifically associated with binocular interaction. No low frequency inhibition of the kind seen in Fig. 1 is found when the adaptation grating is presented to one eye and the test grating to the other⁶. The inhibition of the grating adaptation effect associated with the conditioning grating seems, therefore, to arise from an interaction between the suprathreshold adaptation and conditioning gratings during their simultaneous presentation to different eyes.

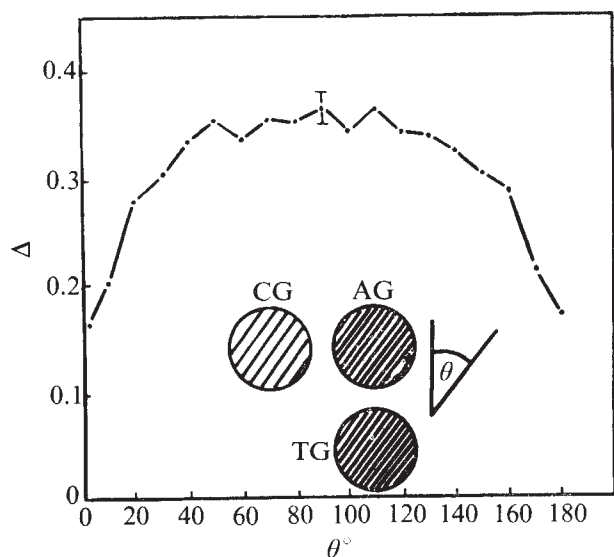


Fig. 3 Values of Δ plotted against θ , the angle at which the gratings were orientated from the vertical. The test and adaptation gratings were of spatial frequency 7.0 cycles per degree and the conditioning grating was of spatial frequency 3.0 cycles per degree. All points are the mean of 10 observations and the vertical error bars denote typical mean errors. The stimulus wavelength was 632.8 nm and the illumination level of the adaptation and conditioning gratings was 4.5 log trolands. $f_{cg} = 3$ cycles per degree; $f_{ig} = f_{ag} = 7$ cycles per degree.

The restriction of the binocular suppression effect to gratings orientated in a vertical direction suggests that it is involved in the stereoscopic fusion mechanism. Suppression of signals from all but matched low frequency components of images presented to the two eyes would facilitate stereoscopic fusion of low frequency elements of the visual field. Investigation of disparity-sensitive neurones in cat and monkey has revealed response features consistent with the characteristics of the binocular interaction described here. Thus, in the monkey, the long axis of the bar-shaped receptive fields of disparity neurones are preferentially orientated vertically², and in the cat, binocular inhibitory interaction is apparent in the responses of such neurones⁸. Stereoscopic fusion effects in human vision associated with periodic structures can be demonstrated more readily for low frequency than for higher frequency structures^{9,10}. The spatial frequency range of inhibitory binocular interaction found in our experiments corresponds approximately to the range of preferred spatial frequencies revealed in these studies on stereoscopic fusion of periodic structures. We have considered the possibility that the observed binocular suppression of the grating adaptation effect is a result of binocular rivalry in perception of the adaptation and conditioning grating stimuli. Neither the spatial frequency specificity of the suppression effect (Fig. 1) nor its restriction to near-vertical orientations of the gratings is, however, characteristic of binocular rivalry observed during our experiments. Other

experiments also indicate that the grating adaptation effect is not modified by binocular rivalry¹¹.

We thank Dr G. J. Burton for helpful discussion and interferometric equipment. We also thank Mr R. Dowdney for help.

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Received December 17, 1975; accepted February 19, 1976.

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Suckling stimulation induces aggression in virgin female mice

THE aggression shown by parturient mammals of various species towards strange conspecifics^{1,2}, referred to as “maternal aggression”, ostensibly serves to protect the young. Although much is known about the aetiology of other forms of aggressive behaviour, no information is available about the mechanism responsible for maternal aggression. We found recently that suckling stimulation may be important in the initiation of aggressive behaviour in parturient mice³. Animals whose nipples were removed before parturition and that were given foster young each day after delivery did not become aggressive. Moreover, we could not induce aggression in virgin female mice by exposing them continuously to foster young. A reason for this may be that virgin females, because of the lack of nipples, which usually develop during pregnancy, did not receive suckling stimulation from foster young. We hypothesised that if nipple growth were induced in virgin females, they would be able to receive suckling stimulation and thus would exhibit aggressive behaviour comparable with that of parturient animals. The results presented here confirm the hypothesis by showing that suckling stimulation induces aggression.

Eighty-five 60–65-d-old virgin female Rockland-Swiss mice (RS) were divided into four groups. One group of 24 mice, after bilateral ovariectomy under ether anaesthesia, received a regimen of hormones that we had found to induce nipple growth. We gave 19 daily subcutaneous injections of 0.02 μ g oestradiol benzoate dissolved in 0.02 ml sesame oil and 500 μ g progesterone in 0.02 ml oil. (The synergistic action of oestrogen and progesterone in mice is confirmed by the fact that little or no nipple growth occurs in response to injections of either oestrogen or progesterone.) Another group of 20 virgin females was ovariectomised and administered oil for 19 d, while another 19 mice were sham ovariectomised and treated daily for 19 d with oil. A group of 22 mice was left intact and mated with RS males. Each animal was housed singly in an 11 $\times$ 7 $\times$ 5 inch polypropylene cage, the floor of which was covered with pine shavings. The animals had free access to food and water and were maintained on a 12 h light/12 h dark cycle with lights on between 0600 and 1800. Testing was between 0700 and 0900 and injections were given between 1000 and 1100.

The virgin mice were tested for aggression 24 h after the final injection of oil or hormone. Animals permitted to mate were tested on day 19 of gestation. An adult RS male was placed into the cage of each animal and left for 3 min or until a fight was observed. A fight was defined as 20 s of continuous biting and chasing. Immediately after the aggression test, each

Table 1 Aggressive behaviour of different groups of females

Group	n	No. fighting before presenting foster young	Proportion (and %) of animals fighting after pup exposure	Latency to begin fighting after pup exposure (d)	Average no. of fights*†
Experiment 1					
Parturient	22	0	12/22 (54)	1.8±0.8	—
OVX+OB+P	24	0	12/24 (50)	1.3±0.6	—
OVX+Oil	20	0	1/20 (05)	1.0	—
S-OVX+Oil	19	0	1/19 (05)	2.0	—
Experiment 2					
Parturient-THEL	20	0	4/20 (20)	1.3±0.4	9.8±0.4
Parturient-S-THEL	20	0	13/20 (65)	1.6±1.1	8.6±2.1
OVX+OB+P-THEL	20	0	2/20 (10)	2.5±1.5	8.0±1.0
OVX+OB+P-S-THEL	20	0	12/20 (60)	2.1±2.4	8.2±2.5

*Animals in experiment 1 were killed after they fought in two successive tests.

†Animals in experiment 2 were given 10 aggression tests. The data are derived from only the animals that fought.

Experiment 1: aggressive behaviour of RS female mice that were either mated (parturient), ovariectomised and primed with oestradiol benzoate and progesterone to induce nipple growth (OVX+OB+P), ovariectomised and treated with oil (OVX+Oil), or sham ovariectomised and given oil (S-OVX+Oil). Aggressive behaviour was scored before exposure to pups (on the last day of hormone or oil treatment or on day 19 of gestation) and on each succeeding day for 10 d after presentation of foster young or until fights were displayed on 2 successive days. Experiment 2: aggressive behaviour of RS females that were mated and either thelectomised (parturient-THEL) or sham thelectomised on day 19 of pregnancy (parturient-S-THEL) and of ovariectomised hormone-treated mice either thelectomised (OVX+OB+P-THEL) or sham thelectomised (OVX+OB+P-S-THEL) on the day after the last hormone injections.

animal was presented with five 3–8-d-old RS foster young. (The pregnant animals were allowed to deliver and their young were removed before presenting them with foster young.) On the next morning each animal was observed for maternal behaviour for 15 min, after which a test for aggression was conducted. Immediately after the aggression test the nipples of each animal were examined (red and distended nipples indicated that the female had received suckling stimulation) and the pups were removed and replaced by five other 3–8-d-olds. This procedure was continued for a further 9 d or until an animal exhibited aggression on two successive tests. The animals were killed at the termination of testing to determine whether milk was present in the mammary tissue.

Every animal exhibited the complete repertoire of maternal behaviour including retrieval of pups, licking of the pups' ano-genital region, and the assumption of the nursing posture. This confirms reports that non-pregnant and non-lactating mice display maternal behaviour⁴.

None of the animals fought when tested before exposure to foster young (Table 1). Instead, they sniffed and groomed the male intruder. After exposure to young, 12 of 22 parturient mice and 12 of 24 hormone-treated virgin females were aggressive. The absence of aggression in approximately 50% of the animals in each of these groups is not unusual for we consistently find that 40–50% of parturient RS females are not aggressive⁵. In contrast, only one of 20 oil-treated, ovariectomised and one of 19 oil-treated, sham-ovariectomised virgins fought. Both oil-treated groups differed significantly from the hormone-treated animals and from the parturient animals with respect to the number that were aggressive (χ^2 , $P < 0.01$).

Aggression typically occurred on the first test after exposure to pups, although a few animals began to fight on the second or third test. The topography of the fighting behaviour of the hormone-treated virgins was indistinguishable from that of the parturient animals. The initial latency to attack averaged 4 s with the attacks consisting of bites to the neck and flanks of the intruder male. Tail rattling and ano-genital sniffing were observed during fights but not before the initial attack. Intruders, never initiating a fight nor fighting back in response to attack, generally assumed submissive postures.

Daily inspection of the parturient and hormone-treated animals after exposure to pups revealed red and distended nipples. In both groups the pups attached themselves to the nipples such that they could maintain contact with the adult when the latter was lifted by the tail. In contrast, red and distended nipples and attachment were never observed in either of

the groups injected with oil. Only the mammary glands of the parturient animals contained milk.

To verify further that the hormone regimen induced nipple growth, six more animals were placed into each treatment group. They were killed either after 19 d of hormone or oil treatment or on day 19 of gestation. The results are summarised in Table 2. On the average, nipple length was 100% and the base diameter was 30% greater for hormone-treated than for oil-treated virgin females. For pregnant animals, the same measures were 200% and 50% greater than those of oil-treated virgins.

Table 2 Average length (mm) and base diameter of a representative nipple of different animals

Group	Average length	Base diameter
Parturient	1.40±0.05*†	0.66±0.06*†
OVX+OB+P	0.99±0.07*	0.55±0.14*
OVX+Oil	0.49±0.08	0.43±0.08
S-OVX+Oil	0.47±0.13	0.41±0.06

Animals were either mated (parturient), ovariectomised and hormonally primed with oestradiol benzoate and progesterone (OVX+OB+P), ovariectomised and treated with oil (OVX+Oil), or sham ovariectomised and given oil (S-OVX+Oil).

*Significantly different (Fisher's least significant difference test, $P < 0.05$) from OVX+Oil and S-OVX+Oil.

†Significantly different from OVX+OB+P.

The findings show that virgin female mice can be induced to show aggression comparable with that of parturient animals by producing nipple growth and presenting them with young. Hormone exposure did not induce fighting directly, for treated animals were never aggressive before exposure to young. Suckling stimulation, therefore, may be essential for the initiation of aggression. To corroborate this we assessed the effect of the suckling stimulus.

Eighty 60–65-d-old virgin female RS mice were divided into four equal groups. Two groups were ovariectomised and given the hormone regimen described above. The nipples of one group were then removed (thelectomy) under ether anaesthesia on the day after the last hormone injection. The other group was sham thelectomised: 1-mm incisions were made along the ventral midline. The two remaining groups were left intact and mated with RS males. One of these groups was thelectomised on day 19 of pregnancy and the other was sham thelectomised. Testing and fostering were as before, except that animals were allowed to complete all 10 aggression tests.

All animals were comparable with respect to maternal behaviour. None was aggressive before exposure to young (Table 1). After exposure to pups, 13 of 20 sham-operated parturient mice and 12 of 20 sham-thelectomised hormone-treated virgins were aggressive compared with four of 20 thelectomised parturient animals and two of 20 thelectomised hormone-treated virgins. The thelectomised groups differed significantly from their sham-operated counterparts with respect to the number that fought ($P < 0.02$). The sham-thelectomised groups did not differ in terms of time taken to become aggressive or the average number of fights exhibited. Daily examination of both sham-operated groups revealed red and distended nipples, indicating that they had received suckling stimulation.

Thus hormone-treated virgins respond to thelectomy in a manner identical to that of parturient mice with thelectomy, significantly reducing the proportion of animals that are aggressive. The data agree with others which demonstrate the attenuating effects of thelectomy on maternal aggression in parturient mice⁹.

Finally, 12 hormone-treated virgins and 12 parturient animals that fought the intruder on the third day of exposure to foster young were deprived of their foster young immediately after the fighting test and were retested 24 h later. If fighting did not occur on retesting, five foster young were placed into the cage and, beginning 24 h later, daily aggression tests and daily fostering of young were carried out until fighting recommenced. Animals that fought 24 h after removal of foster young continued daily aggression tests in the absence of the young. As soon as fighting was no longer exhibited, foster young were reintroduced and daily tests were conducted until fighting began.

All animals stopped fighting after pups were removed and resumed fighting when they were replaced. The hormone-treated virgins and the parturient animals did not differ in terms of the number of days after removal required to terminate fighting (range = 1–4 d, median = 1.0 d) or the number of days of pup replacement required to re-establish fighting (all within 1 d). Each animal had red distended nipples and nipple attachment after replacement of foster young. Thus the aggression of both hormone-treated and parturient mice can be eliminated by removing the suckling stimulus and re-established by reintroducing that stimulus.

The fighting behaviour induced in female mice hormonally primed to induce nipple growth could have been a response to chronic stress, produced by chronic suckling by deprived young, rather than a response specific to the suckling stimulus. We do not believe that this is the case, however, because virgin females and lactating mice whose pups had been removed did not attack a male after 24 h of intermittent electric shock to the paws. Moreover, virgins and lactating mice deprived of young did not attack a male after chronic application of painful stimulation to the nipples. Thus fighting in hormone-treated virgin female mice seems to be a response specific to the suckling stimulus.

Our data show that suckling not only supplies nourishment to the young but also affects the relationship between the mother and other adult members of her species. Suckling stimulates the release of hypothalamic and pituitary hormones⁶, alters the rate of synthesis of hypothalamic monoamines⁷, and changes the firing patterns of hypothalamic neurones⁸. One or more of these changes may induce aggressive behaviour in lactating mice.

This research was supported by grants from the US Public Health Service.

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Transmission of *Trypanosoma rotatorium* from frogs to white mice

ALTHOUGH the trypanosome *Trypanosoma rotatorium* Mayer 1843, lives in the blood of frogs (*Rana esculenta* and *R. temporaria*) it does not have a pathogenic effect on them. Since no information is available about the mass incidence of these parasites in the blood of homeothermic animals, we have tried to infect white mice with *T. rotatorium* in order to study the development of infection in these hosts.

Our experimental group consisted of 100 male and female white laboratory mice, strain ICR, from 3 weeks to 3 months old, which had been standardised both microbiologically and parasitologically (specific pathogen-free). Blood from young frogs (0.4–0.6 ml) diluted in 0.9% NaCl was administered intraperitoneally so that the final infective dose was 500 trypanosomes per animal. For controls we used 50 mice aged 3 weeks to 3 months which were given blood from uninfected frogs diluted in saline (2:1 v/v); 25 of them were injected with 2.5 mg of hydrocortisone twice a week for 3 weeks. The criterion for termination of incubation was the first appearance of trypanosomes in the peripheral blood of the mice. Dry blood smears were stained by the standard Giemsa method.

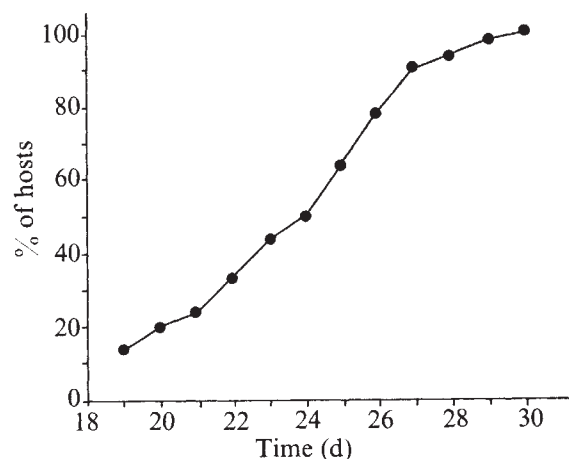


Fig. 1 The first incidence of *Trypanosoma rotatorium* in the blood of the individual mice is dependent on time.

Infection developed slowly with an incubation period of 18–30 d. There was considerable variation in the first appearance of trypanosomes in individual mice (Fig. 1). On day 24 of infection, trypanosomes were present in the blood of 50% of mice, 6 d later they were present in all hosts (100 mice).

The slender body of the trypanosomes, 10.6–18.7 μ m long and 1.3–5.0 μ m wide, ended in a short, obtuse rostrum. The oval nucleus was in the middle of the body and the blepharoplast was near its end. The undulating membrane passed into a short, free flagellum whose length did not exceed one-tenth of the body length (Fig. 2). Numerous dividing trypanosomes appeared in the blood of the mice. Division was binary and of the standard type. Multiple division in crithidia was not observed (Fig. 3).

No overt signs of disease were evident before the first appearance of trypanosomes in the blood of infected animals. An increase in the number of blood parasites was manifest in

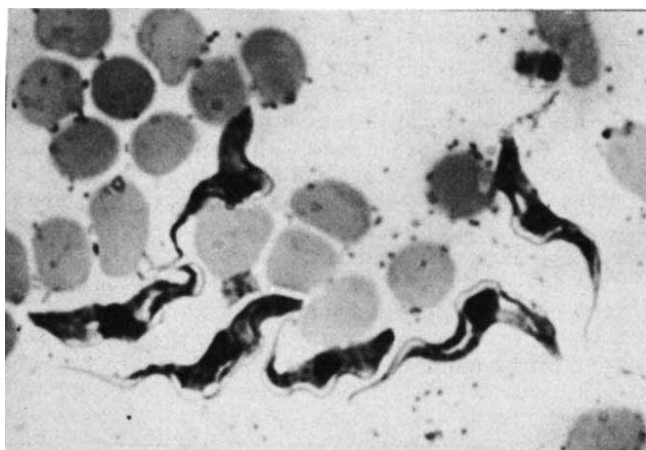


Fig. 2 *T. rotatorium* in the blood of a white laboratory mouse. Dry smear stained with Giemsa ($\times 1,600$).

loss of appetite, loss of weight, bristling of the fur and general restlessness. The animals died within 2–5 d of multiplication of the parasites in the blood (maximum incidence 100,000 trypanosomes per ml of blood).

Since no information was available about transmission of trypanosomes from poikilotherms to homoeotherms our findings with mice had to be compared with published data on parasites from tadpoles and frogs^{2–6}. During the development of infection in the frog, the morphology of trypanosomes changes considerably^{2–6}. Trypanosomes from tadpoles and young frogs are slender, with a long flagellum^{5,6}. Those multiplying in the blood of mice, however, reached about half the size of those multiplying in frog blood.

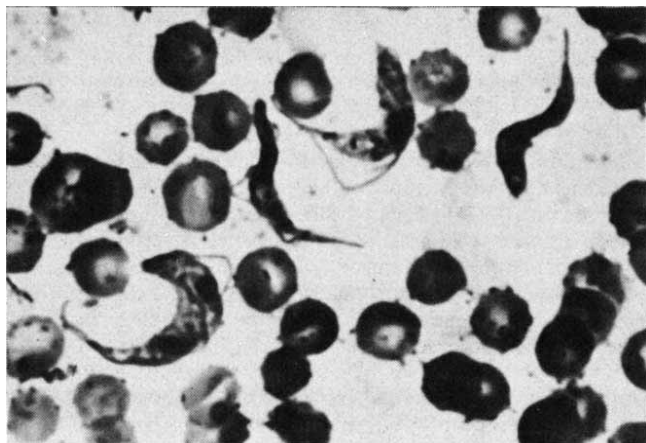


Fig. 3 In the left corner at the bottom *T. rotatorium* dividing in blood of white mouse. Stained with Giemsa ($\times 1,600$).

A form of trypanosome that is often described from the blood of adult frogs is large and almost immobile, with a striped pellicle and a short undulating membrane which does not continue in a free flagellum^{2,4–6}. This form has never been found in mice.

Compared with *T. duttoni*, one of the most frequent trypanosomes of rodents^{1,7}, *T. rotatorium* from mice was more slender, and its rostrum was shorter. There were differences in the location of the blepharoplast which is near the nucleus in *T. duttoni*, and the length of the free flagellum which reaches a half to two thirds of the body length of *T. duttoni*. In contrast to *T. duttoni*, *T. rotatorium* never produced multiple forms in mouse blood^{1,7}.

Thus the morphology of trypanosomes from mice is closest to that of trypanosomes from tadpoles and young frogs, except

for the larger body size of those in the poikilothermic hosts and the very short free flagellum of those in mice.

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Received December 16, 1975; accepted February 25, 1976.

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Study of haemopoietic microenvironment *in vitro*

THE concept of the haemopoietic inductive microenvironment as first proposed by Curry and Trentin¹ has been amply confirmed by experiment. One salient feature of this concept relates to the role of the mouse spleen in promoting erythropoiesis rather than granulopoiesis. For example, in the clonal growth of transplanted bone marrow cells, the spleens of the irradiated host mice have erythroid and granuloid colonies in a ratio of 3, whereas the ratio is 0.5 in the bone marrow of the same hosts^{2,3}. Other data show that there is a positive effect of splenic tissue, rather than a negative effect of bone marrow, on erythroid colony growth³. We have tested this concept using cocultures of mouse spleen and bone marrow cells and have found that

Table 1 Effect of NS and XS cells on marrow erythropoiesis

	Haemoglobin ⁵⁹ Fe ($\pm$ s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells—E	17 $\pm$ 6	—	100
Marrow cells+E	232 $\pm$ 16	215	1,260
NS cells—E	3 $\pm$ 2	—	18
NS cells+E	17 $\pm$ 3	14	100
Marrow+NS cells—E	50 $\pm$ 10	—	294
Marrow+NS cells+E	286 $\pm$ 45	236	1,680
XS cells—E	0	—	0
XS cells+E	0	0	0
Marrow+XS cells—E	150 $\pm$ 11	—	880
Marrow+XS cells+E	491 $\pm$ 18	341	2,890

E, erythropoietin. Cells were cultured in 0.2 ml samples, as previously described⁶, in the wells of culture dishes. The medium, pH 6.9, consisted of 65% NCTC-109 containing 30 mM morpholinopropane sulphonic acid (MOPS), 30% foetal calf serum, 5% mouse serum containing 73 nmol of ferric nitrate per ml, and gentamicin (0.5 mg ml⁻¹). Both the foetal calf serum and the mouse serum were heat-inactivated at 56 °C for 30 min before use. Six replicate cultures were established per group. A sheep plasma preparation of erythropoietin (16 mU) with a potency of 200 U per mg of protein was added to the appropriate cultures dissolved in 1 μ l of 0.1% bovine serum albumin, 0.15 M NaCl. The wells were sealed with sterile plastic adhesive sheets. After 20 h of incubation at 37 °C the wells were opened and 20 μ l of ⁵⁹Fe-labelled mouse serum (0.2 μ Ci) were added to each; the wells were then resealed and incubated for 5 h, after which incorporation into haemoglobin was measured as before^{6,7}. Spleens were removed aseptically from 10–14-week-old BDF₁ female mice. XS cells were prepared from mice which had received 900 R of whole-body X rays 3 d before use. Marrow cells were cultured at 2.9×10^6 per well; NS and XS cells were cultured at 2.7×10^6 per well. Spleen cells were dispersed by forcing splenic tissue through a sterile stainless steel screen (60 mesh) into 70% NCTC-109, 30% foetal calf serum. Remaining cell clumps were broken up by repeated passage of the suspension through a 26-gauge needle. The cells were washed once in phosphate-buffered saline before appropriate dilution in complete medium. Essentially the same results were obtained when the spleen cells were dispersed by digestion with collagenase.

Table 2 Effect of freezing and thawing of irradiated spleen cells

	Haemoglobin ⁵⁹ Fe ($\pm$ s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells - E	5 $\pm$ 3	—	100
Marrow cells + E	112 $\pm$ 15	107	2,240
XS cells - E	0	—	0
XS cells + E	0	0	0
Marrow + XS cells - E	52 $\pm$ 2	—	1,040
Marrow + XS cells + E	197 $\pm$ 14	145	3,040
Frozen-thawed XS cells - E	0	—	0
Frozen-thawed XS cells + E	0	0	0
Marrow + frozen-thawed XS cells - E	19 $\pm$ 3	—	380
Marrow + frozen-thawed XS cells + E	85 $\pm$ 14	66	1,700

Culture conditions and procedure were as outlined in Table 1. In the preparation of frozen and thawed XS cells, the cells in 25 μ l of complete medium were added to appropriate wells and the dishes were placed on a mixture of crushed dry ice and ethanol for 15 min. They were then incubated at 37 °C for 20 min before the bone marrow cells were added in 175 μ l of complete medium. Marrow cells were cultured at 3.2×10^6 per well; XS cells were cultured at 1.7×10^6 per well.

phenomena related to the haemopoietic inductive micro-environment can be studied *in vitro*.

Table 1 shows that spleen cells in culture augment haemoglobin synthesis by marrow cells *in vitro* in two ways. These may be characterised as an effect on baseline haemoglobin synthesis and an effect on erythropoietin-induced haemoglobin formation. Cells from the spleens of normal mice (NS cells), as well as from the spleens of mice given 900 R of whole-body X radiation 3 d earlier (XS cells), caused increased incorporation of ⁵⁹Fe into haemoglobin when they were added to marrow cell cultures. The effect on the baseline was almost three times greater with XS than with NS cells. Neither NS nor XS cells alone synthesised significant amounts of haemoglobin by the method used. In addition, XS cells but not NS cells caused a significant potentiation of the marrow cell response to erythropoietin, amounting to about 60% more stimulated haemoglobin synthesis than found with marrow cells alone. The NS cells showed a slight response to erythropoietin, but XS cells never did.

We attempted to determine whether intact XS cells were required for the observed effects. The data in Table 2 show that freezing and thawing of the cells reduced but did not abolish the effect on the baseline, and completely eliminated the effect on erythropoietin-induced haemoglobin synthesis. In fact, the frozen-thawed preparation caused a 38% suppression of the response to erythropoietin (Δ c.p.m.). This suppression is significant at the $P < 0.02$ level.

Table 3 Effect of further irradiation of spleen cells on marrow haemoglobin synthesis

	Haemoglobin ⁵⁹ Fe ($\pm$ s.d.) (c.p.m.)	Δ c.p.m.	% of marrow control
Marrow cells - E	8 $\pm$ 1	—	100
Marrow cells + E	77 $\pm$ 5	69	963
XS cells - E	0	—	0
XS cells + E	0	0	0
Marrow + XS cells - E	40 $\pm$ 3	—	500
Marrow + XS cells + E	155 $\pm$ 7	115	1,940
XXS cells - E	0	—	0
XXS cells + E	0	0	0
Marrow + XXS cells - E	40 $\pm$ 2	—	500
Marrow + XXS cells + E	170 $\pm$ 11	130	2,120

Culture conditions and procedure were as outlined in Table 1. The XXS cells were prepared by irradiation of a sample of the XS cell suspension with 10,000 R of X rays just before culturing. There were 3.2×10^6 marrow and 1.8×10^6 spleen cells per well.

Additional X irradiation of XS cells *in vitro* with 10,000 R (XXS cells), immediately before they were added to the marrow cell cultures, enhanced the response to erythropoietin to a significantly greater extent ($0.02 > P > 0.01$) than did the single *in vivo* irradiation (Table 3). The effect on baseline haemoglobin synthesis, however, was the same with both XS and XXS cells, amounting to five times more incorporation.

The two effects (enhanced baseline synthesis and potentiation of response to erythropoietin) were distinguished further when species specificity was studied. Table 4 shows that baseline haemoglobin synthesis by rat bone marrow cells was increased 49% by mouse NS cells and almost 200% by mouse XS cells. In contrast, the response of rat marrow to erythropoietin was not affected by mouse NS cells and was depressed by mouse XS cells.

Table 4 Effect of mouse spleen cells on rat marrow erythropoiesis

	Haemoglobin ⁵⁹ Fe ($\pm$ s.d.) c.p.m.	Δ c.p.m.	% of marrow control
Rat marrow cells - E	47 $\pm$ 11	—	100
Rat marrow cells + E	182 $\pm$ 14	135	387
Mouse NS cells - E	0	—	0
Mouse NS cells + E	0	0	0
Rat marrow + mouse NS cells - E	70 $\pm$ 7	—	149
Rat marrow + mouse NS cells + E	202 $\pm$ 15	132	430
Mouse XS cells - E	0	—	0
Mouse XS cells + E	0	0	0
Rat marrow + mouse XS cells - E	140 $\pm$ 9	—	298
Rat marrow + mouse XS cells + E	226 $\pm$ 18	86	481

Culture conditions and procedure were as outlined in Table 1, except that bone marrow cells from male Sprague-Dawley rats (250–275 g) were used and rat serum was substituted for mouse serum. Marrow cells were cultured at 3×10^6 per well; NS and XS cells were cultured at 1.8×10^6 per well.

The data presented here permit several conclusions with respect to an understanding of the haemopoietic inductive micro-environment. (1) The role of the spleen in promoting erythropoiesis of mouse bone marrow cells can be studied *in vitro*. (2) Two different mechanisms seem to augment haemoglobin synthesis by splenic tissue. One concerns the existing level of haemoglobin formation by normal marrow cells; the second concerns the increase in response to added erythropoietin. (3) The action of normal spleen cells on baseline haemoglobin synthesis can be duplicated partially by cells which have been frozen rapidly and thawed, making it likely to be an effect of a cell constituent rather than of viable cells. (4) Mouse spleen cells can affect the baseline haemoglobin synthesis of rat marrow cells. (5) The action of either normal spleen cells or cells from the spleens of irradiated mice potentiates the action of erythropoietin on haemoglobin synthesis, but this effect is restricted to intact cells and may be species specific. Although it might be argued that the extent of erythropoietin-stimulated haemoglobin synthesis is maximal when rat marrow cells are incubated with mouse XS cells, so that an increase in the baseline will cause a necessary decrease in the erythropoietin effect, we have seen, in other experiments, much greater induced haemoglobin synthesis. This observation makes it more plausible that the decrease in c.p.m. from 135 to 86 (Table 4) represents a true suppression of erythropoietin action.

The nature of the cell constituent postulated above to be responsible for the action of spleen cells on baseline haemoglobin synthesis is unknown, and will be investigated further. Our evidence suggests that the second of the effects, that of potentiation of the response to erythropoietin, is mediated by intact cells. Bessis and Breton⁴ have described a large cell,

resembling a macrophage, around which erythroblasts are clustered. They have proposed that this cell acts as a "nurse cell" in normal erythropoiesis by facilitating iron transfer to the developing erythroblasts. This type of cell might be responsible for the effect we describe. Alternatively, the effect might be due to the type of radio-resistant cell that is found in spleen and is involved in the processing of antigen in the immune response⁶.

We thank Nancy Pech for technical assistance. This work was supported in part by grants from the National Cancer Institute. G. E. v. Z. holds a National Institutes of Health postdoctoral research fellowship. The Franklin McLean Memorial Research Institute is operated by the University of Chicago for the US Energy Research and Development Administration under contract.

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Several proliferative phases precede maturation of IgG-secreting cells in mitogen-stimulated cultures

SEVERAL workers have shown that stimulation of mouse spleen lymphocytes by *E. coli* lipopolysaccharide (LPS) results in extensive proliferation of B lymphocytes and the rapid maturation of immunoglobulin M (IgM)-secreting cells¹⁻³. Not all B lymphocytes that are stimulated to DNA synthesis and division by LPS, however, develop into high rate Ig-secreting cells^{4,5}; dividing blast cells can give rise to large numbers of non-dividing small B lymphocytes with a high density of surface Ig and no detectable intracellular Ig pool⁴. In these respects such secondary cells are thought to resemble memory cells. Those cells which do develop into high rate Ig-secreting cells during a few days of culture are generally restricted to secretion of IgM^{7,8}. We report here that in improved culture conditions, and dependent on repeated cycles of mitogenic stimulation, precursors to IgG and IgA as well as IgM-secreting cells can undergo the full maturation sequence. Our results suggest that one or more phases of resting cells are intermediates in this sequence.

Mouse spleen lymphocytes were stimulated with LPS in

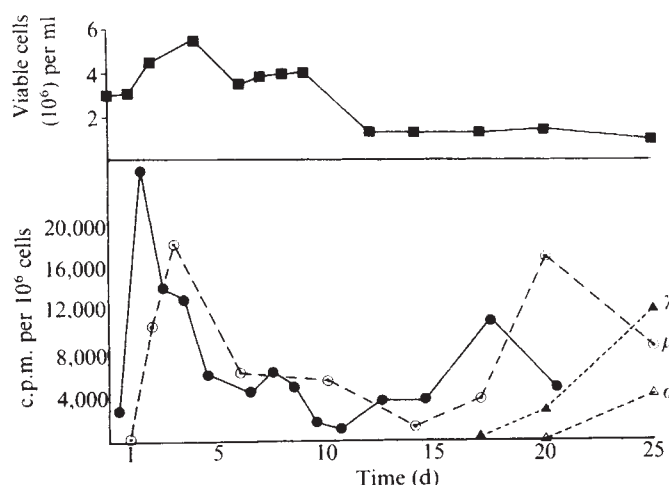


Fig. 1 DNA synthesis and Ig secretion in LPS-stimulated cultures. Spleen cells (CBA mice) were suspended, washed once and cultured at 3×10^6 cells per ml in RPMI 1640 medium (Biocult) supplemented with LPS (*E. coli* 055B, Difco, $10 \mu\text{g ml}^{-1}$) 10% foetal calf serum (Flow), antibiotics and 6×10^{-5} M 2-mercaptoethanol (2ME). Culture dishes (60 mm) containing 5 ml were kept stationary at 37°C in an atmosphere of 10% CO_2 , 7% O_2 and 83% N_2 . Every 3 d cells were sedimented (5 min at 240g) and resuspended in fresh, full medium and LPS. For ^3H -thymidine incorporation, duplicate 0.2 ml samples from the above cultures were incubated for 24 h at 37°C with $1 \mu\text{Ci}$ ^3H -thymidine (50 mCi mmol^{-1}) in microplates. The cells were filtered on Whatman glass fibre circles (GF/A), and washed with phosphate-buffered saline, trichloroacetic acid, ethyl alcohol and ether⁹. Assessment of thymidine incorporation on microscale is justified; 4-h pulses in more recent experiments yield analogous results. For Ig secretion, cells sedimented from 0.6 ml of culture were incubated for 4 h in 0.4 ml of RPMI medium (containing only 2.5×10^{-5} M methionine), 10% foetal calf serum, 6×10^{-5} M 2ME and $20 \mu\text{Ci}$ ^{35}S -methionine ($>100 \text{ Ci mmol}^{-1}$). The medium was then diluted with phosphate-buffered saline and methionine (1 mg ml^{-1}), cells were removed and the supernatant was clarified by high speed centrifugation. Ig was precipitated from half the supernatant using a polyvalent rabbit antiserum to mouse IgM and IgG and carrier Ig^{8,9}. Ag/Ab precipitates, washed twice, were reduced and analysed by polyacrylamide gel electrophoresis as described in Fig. 2.

the conditions described in the legend to Fig. 1. It is important for the long term maintenance of these cultures that medium be replaced every 3 d. The increase and then decline in cell number as well as the pattern of ^3H -thymidine incorporation and Ig secretion during 25 d are shown in Fig. 1. A high rate of ^3H -thymidine incorporation is observed on days 2 and 3 and followed on day 7 and again on day 17 by additional peaks of incorporation. Increases in cell numbers on days 4 and 8 reflect these high thymidine incorporation rates. The rate of Ig secretion increases to a maximum on day 3, after which it falls to a relatively low level. By day 20 the rate of Ig secretion is again very high (Fig. 1).

We have analysed the class of Ig secreted at various times in culture by fractionation of the Ig heavy chains on sodium dodecyl sulphate (SDS)-polyacrylamide gels.

Table 1 Induction of IgG secretion in T-cell depleted spleen

Spleen donor		Radioactivity (c.p.m. ml^{-1})†			Viable cells per ml ($\times 10^{-6}$)	
		Day 19	28	31	19	28
Normal	μ Chains	8,470	38,060			
	γ Chains	2,730	20,620		7.0	1.5
T $\times$ B*	μ Chains	12,540	34,140	33,490		
	γ Chains	2,640	10,470	12,900	6.8	2.1

* (CBA $\times$ C57)F₁ mice were thymectomised, irradiated with 850 r. and reconstituted with foetal liver for 10 weeks. Spleen cells were treated with AKR anti-C₃H Thy 1.2 and complement before culture¹¹.

† Radioactivity of secreted Ig heavy chains (^{35}S -methionine labelled) was analysed by electrophoresis after reduction of Ig precipitated with antiserum (see Fig. 2).

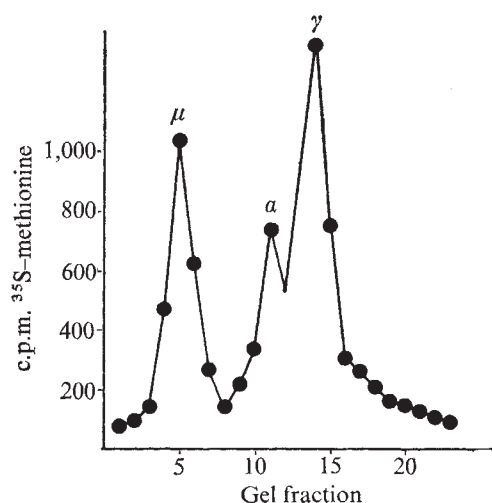


Fig. 2 Fractionation of secreted immunoglobulin heavy chains. ^{35}S -Ig secreted on day 25 (see legend to Fig. 1) was precipitated with antiserum to Ig, dissociated in 2% SDS, reduced overnight in 0.15 M 2ME at 37 °C in borate-buffered saline, and alkylated with iodoacetamide for 1 h at pH 8. Polyacrylamide gel electrophoresis was carried out as described by Maizel¹⁰, gels were dried and then cut into 2-mm strips which were prepared for liquid scintillation counting by treatment with 0.5 ml H_2O_2 for 3 h at 70 °C. Internal markers for μ and γ chains were included on every gel and revealed by staining with Coomassie blue. In addition, labelled heavy chains of myeloma proteins confirmed the position of μ , γ and α chains.

A gel profile of reduced and alkylated Ig secreted into the culture medium during a pulse of ^{35}S -methionine on day 25 is shown in Fig. 2. Analysis on different days shows that only immunoglobulin of the IgM class is secreted early in the culture period, while late immunoglobulin secretion includes large amounts of IgG, some IgA and a new wave of IgM (Fig. 1). Cells fixed and stained for intracytoplasmic Ig using class-specific and fluorescent antisera (prepared by R. M. E. Parkhouse) have confirmed the presence of cells synthesising each of these Ig classes (unpublished results of M.Z. and R. M. E. Parkhouse).

Neither the activation of precursors nor the lag in maturation of the IgG-secreting cells depends on the presence of T cells, as shown by the similarity of results obtained using spleens from mice depleted of T cells, both *in vivo* and *in vitro*, or from normal mice (Table 1). The difference in amount of IgG secreted in the two types of culture is within the range of variation normally observed with different pools of spleen cells.

It has been shown¹² that the stimulation by antigen of cells producing specific IgG antibody in short term culture depends on the presence of a transient cell type which appears to be an intermediate between a long term memory cell and an antibody-producing cell. We have investigated whether this intermediate cell type responds to LPS and

whether a transient pool of such cells in a normal spleen might account for the IgG response we have observed. Table 2 shows that these cells do respond to LPS and give comparable numbers of antibody-producing cells as in antigen-stimulated cultures. The peak of this response, however, is on day 5 and is easily differentiated from the larger LPS-stimulated IgG response detected by isotope incorporation and gel analysis on day 17. It is likely that this intermediate cell type is responsible for the reported^{14,15} stimulation of a small number of IgG antibody-forming cells by mitogen in short term cultures.

The occurrence of a long lag period in LPS-stimulated cultures during which the level of ^3H -thymidine incorporation is low suggested that a non-proliferating cell might be an intermediate in the maturation of precursors to IgG and other late Ig-secreting cells. This was further investigated by the addition of bromodeoxyuridine (BUdR) to cultures at various times at a concentration ($10\text{ }\mu\text{g ml}^{-1}$) which on subsequent irradiation by means of a black lamp (300–400 nm), leads to the death of cells actively engaged

Table 3 Effect of BUdR on IgG secretion

Days	c.p.m. per ml culture* ^3H -thymidine	Cell no. per ml $\times 10^{-6}$	Ig† c.p.m. per ml culture μ chain	γ chain
Control 4	35,500	6.7	56,071	< 500
Control 7	9,250	3.0	54,417	4,455
Control 14		0.8	14,797	3,869
BUdR‡ 4	2,700	3.5	25,344	7,216
BUdR 7	48,250	1.9	4,356	< 440
BUdR 14		3.3	43,026	27,112

* 24-h pulse with $1\text{ }\mu\text{Ci } ^3\text{H}$ -thymidine (50 mCi mmol^{-1}).

† Radioactivity of Ig secreted during a 4-h pulse with ^{35}S -methionine (see legends to Figs 1 and 2).

‡ BUdR ($10\text{ }\mu\text{g ml}^{-1}$) was added on day 3; after 24 h cells were collected and irradiated in the same volume as before in PBS for 20 min using a black lamp (Philips 20 W/08, 300–400 nm) before being resuspended in fresh medium. The same dishes were used throughout to avoid loss of adhering cells. Control cultures were handled in identical fashion in absence of BUdR.

in DNA synthesis. Only a few cells survived (less than 5%) when BUdR was present from 36 to 62 h after stimulation during the first wave of DNA synthesis, indicating that the dividing cells were killed. Precursor cells, however, did survive when a BUdR pulse was given on days 4 and 5 or 10 and 11, as shown by the maturation of large numbers of Ig-secreting cells later in the culture period (see below). It should be emphasised in this connection that stimulation of high rate IgG secretion depends on the re-addition of LPS to cultures when they are resuspended in fresh medium.

An unexpected finding was that if BUdR is added and cultures are irradiated immediately after the first peak of ^3H -thymidine incorporation, precursor cells which survive initiate high rate IgG secretion and do so earlier than do controls. LPS-stimulated cells received a 24-h pulse of BUdR on day 3 of culture. ^3H -thymidine incorporation on day 4 immediately after the pulse is very low. By day 7, however, it has increased to a level many times greater than that observed in controls (Table 3). This effect is not due to a change in the specific activity of intracellular pools as a considerable increase in cell numbers occurs in parallel. Analysis of Ig secretion showed some early IgG (day 4) and large amounts of IgG on day 14. In these control cultures (washed and irradiated in the absence of BUdR) detectable IgG appeared earlier than usual (Table 3). Our interpretation of these experiments is that an inhibitory cell type which proliferates in LPS-stimulated cultures is eliminated by irradiation after the BUdR pulse. Secondary non-proliferating precursors quiescent during the pulse can then be activated sooner than would otherwise occur. Other experiments showed that cells present on day 9 of culture

Table 2 Specific anti-DNP-forming cells and IgG secretion

Additions	Day 5	7	10	13	17
	7S PFC/* culture				
None	530	20	20	20	—
DNP-KLH } ($0.1\text{ }\mu\text{g ml}^{-1}$) }	3,360	1,080	170	40	0
LPS	2,300	300	620	545	30
	Secreted IgG (c.p.m. per culture)				
LPS	< 800	920	790	1,380	9,530

PFC, Plaque-forming cells. Radioactivity of secreted ^{35}S -methionine-labelled IgG was estimated as in Figs 1 and 2. Spleen cells were derived from mice primed and then boosted 8 d previously with DNP-KLH (dinitrophenol-keyhole limpet haemocyanin)¹². Culture conditions were as described in Fig. 1.

* Specific indirect anti-DNP-forming cells were detected using horse red blood cells sensitised with trinitrobenzene sulphonic acid and then developing plaques with rabbit anti-mouse IgG^{12,13}.

could block the activation of precursors to early IgM-secreting cells when mixed with freshly prepared spleen cells before, but not 27 h after, addition of LPS (M.Z., unpublished). Nevertheless, in view of the complexity of BUdR action^{16,17}, we cannot exclude the possibility that BUdR has a direct effect on precursor cells which speeds up further proliferation or leads to their rapid maturation. As all cells which are engaged in DNA synthesis are killed at the end of the pulse, this would have to be an effect on cells not proliferating during the pulse.

There have been two recent reports^{5,18} that LPS treatment results, within 6–8 d, in the appearance of cells which synthesise IgG as detected by immunofluorescent staining of cytoplasmic Ig with specific antisera. In one case⁵, these are described as immature, weakly staining lymphoblasts, while in the other¹⁸, stronger staining denotes a more mature cell type in cultures initiated at low density. At such early times we detect low levels of IgG secretion in low density cultures set up according to Kearney and Lawton¹⁸ and occasionally also in the conditions described here. High levels of IgG production, however, arise only late in our cultures and it is possible that several mitotic cycles are required before full maturation of these cells. The significance of initiating cultures at a low cell density is not clear, but may be a means of reducing inhibitory effects.

In conclusion, we have shown that precursors to IgG and IgA, as well as to IgM-secreting cells, can be activated by a mitogenic stimulus. After a proliferative phase they give rise to early IgM-secreting cells as well as to secondary non-proliferating cells. These secondary cells can be re-activated to undergo further proliferation and finally to mature to high rate IgG-, IgA- or IgM-secreting cells. An inhibitory cell type which also proliferates in these high density cultures may delay the reactivation of secondary cells. Neither the maturation of precursors to IgG production nor the inhibitory effects observed in LPS-stimulated cultures depends on the presence of T cells.

We thank P. Tilly for assistance and J. North for stimulating discussions.

Note added in proof: Additional evidence of production of IgG- or IgD-like molecules in low density cultures has been published¹⁹.

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Received November 24, 1975; accepted February 26, 1976.

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of individual complement components (C), from both the classical and the alternative pathways, are influenced by genes located close to the major histocompatibility complex (MHC), (reviewed in ref. 2). The relationship between mouse Ss protein, serum levels of which are controlled by a gene in the central part of *H-2*, and human C4 (refs 3–5), highlights the association between MHC and the C system. The mechanism which underlies this association is largely unknown. In only one case (factor B) has a structural gene been identified⁶. We report here that the serum levels of C3 in mice during ontogeny are influenced by a gene in the central region of the *H-2* complex.

Breeding couples of inbred, congenic and congenic recombinant strains of mice were obtained either from commercial sources, or from Drs D. C. Shreffler and J. Stimpfling. Mice were bled in heparin-treated capillary tubes and plasma was stored at -70°C . Antiserum to mouse C3 was obtained as before⁷. Immunoelectrophoretic analysis showed that this antiserum did not distinguish between native C3 and its degradation products C3b and C3d obtained by treating mouse serum with cobra venom factor. The specificity of this antiserum was ascertained by comparison with a monospecific antiserum to guinea pig C3 which cross reacted with mouse C3. In double diffusion in agarose against mouse serum, the lines given by the rabbit antiserum to guinea pig C3 and to mouse C3 coalesced in a reaction of identity. Simple radial immunodiffusion was performed as before⁸. Four different dilutions of a pool of normal mouse plasma were included in each plate as standards. Plates were incubated for 7 d at room temperature. When linear regressions were calculated for different plates for the relationship between areas of the rings compared with dilution of the standard antigen, coefficients of 0.999 or higher were obtained.

C3 levels in plasma of AKR/J mice were significantly higher than in DBA/2J until 4 weeks of age (ref. 8 and Table 1). At 9 weeks the differences were smaller and not significant. To assess the importance of the *H-2* complex in the control of C3 levels during development, segregation analysis and studies were performed with congenic and congenic recombinant mice which were 2 and 3 weeks old.

Table 1 summarises the results of segregation analysis, some of which have been reported already⁸. The 21-d-old progeny of the backcrosses (AKD2F₁ ♀ × DBA/2J or AKR/J ♂) or of the F₂(AKD2F₁ × AKD2F₁) mice were typed for *H-2* and classified as *k/k*, *d/k* or *d/d*. In all, 203 mice were examined. C3 levels were compared in all pertinent combinations and significant differences ($P < 0.01$) in the expected direction were found in mice with different *H-2*. Furthermore, the differences in C3 levels between AKR/J and DBA/2J mice seem to be controlled by very few genes, for, with a single exception, there were no significant differences in C3 levels of mice with the same *H-2* type (Table 1). In ten comparisons, the only exception was that C3 levels of DBA/2J mice were significantly lower than those of the *d/d* progeny of F₂ mice. Measurements (not shown) of C3 levels at 15 d of age in the same backcrosses and F₂ showed an identical pattern of segregation. Body weights of mice with different *H-2* at 2–3 weeks of age were not different.

Table 2 shows that differences in C3 levels were detected among 21-d-old congenic mice of the B10 series which bear five unrelated *H-2* haplotypes. Plasma from 142 animals was analysed. *H-2^b* and *H-2^d* haplotypes are associated with small but significant differences in C3 levels. As expected from the results of the segregation analysis, *H-2^k* mice have significantly higher C3 levels than *H-2^d* mice. No differences were found between C3 levels of mice with *H-2^k*, *H-2^s* and *H-2^f* haplotypes.

The effect of allelic variation within the subregions of the *H-2* complex was studied in series of congenic and congenic recombinant mice with different S subregions of *H-2*. Each group consisted of a minimum of 20 mice, from three or more separate litters. Table 3 shows that C3 levels in A.AL strain were higher than in A strain. They differed only in the IC and S subregions. A.AL carries the *k* allele, and A carries the *d* allele in those subregions. In the C3H series, C3H.OH(S^d) and

Control of C3 levels in mice during ontogeny by a gene in the central region of the *H-2* complex

THE observation that differences in serum haemolytic activity in mice are controlled by the *H-2* complex¹, was followed by reports that in several mammalian species the levels

Table 1 Segregation of C3 levels in serum of 21-d-old mice according to *H-2*

<i>H-2</i> type	AKR/J	DBA/2J	F ₁	F ₁ × AKR/J	F ₁ × DBA/2J	F ₁ × F ₁	F _y ^x
<i>k/k</i>	0.86 ± 0.13 (29)	—	—	0.81 ± 0.09 (8)	—	0.91 ± 0.14 (12)	F ₄₆ ² = 1.57 NS
<i>d/k</i>	—	—	0.70 ± 0.07 (8)	0.74 ± 0.06 (17)	0.69 ± 0.09 (14)	0.76 ± 0.12 (45)	F ₈₀ ³ = 2.58 NS
<i>d/d</i>	—	0.53 ± 0.06 (36)	—	—	0.58 ± 0.09 (12)	0.68 ± 0.10 (22)	F ₆₇ ² = 23.4 P < 0.01
F _y ^x	F ₇₀ ² = 100 P < 0.005		F ₂₃ ¹ = 53 0.025 > P > 0.01		F ₂₄ ¹ = 9.9 P < 0.005	F ₇₆ ² = 15.0 P < 0.005	

Numbers not in parentheses represent relative concentration of C3 in plasma (mean ± s.d.). Numbers of animals are given in parentheses. The differences or similarities in complement levels between groups of strains, or multiple groups of animals, were estimated by analysis of variance. In the F_y^x ratios, x represents degrees of freedom for genetic variation and y represents degrees of freedom for phenotypic variation.

C3H.OL(S^k) strains, which differ only in the S subregion, have small but significantly different C3 levels in the direction predicted from the segregation studies. B10.Br and B10.M(17R) are identical in the K, IA and IB subregions, and have different C3 levels; B10.Br is *k* in the IC, S and D subregions, and has higher C3 levels than B10.M(17R) which is *d* in IC and S subregions.

These comparisons show that the gene controlling C3 levels

Table 2 C3 levels in congenic mice with different *H-2* haplotypes

Congenic line	<i>H-2</i>	C3 levels
C57BL/10sn	<i>b</i>	0.81
B10.D2	<i>d</i>	0.86
B10.M	<i>f</i>	0.95
B10.Br	<i>k</i>	0.96
B10.S	<i>s</i>	0.98

H-2^b against *H-2^d* ($F_{63}^1 = 6.8$; $0.05 > P > 0.01$). *H-2^b*, *H-2^k* and *H-2^s* against *H-2^b* and *H-2^d* ($F_{140}^2 = 40.6$; $P < 0.001$). The differences between *H-2^k*, *H-2^f* and *H-2^s* are not significant.

is located within or closely linked to the S subregion of *H-2*. Some additional observations are compatible with this hypothesis. (1) In the A/WySn-A.CA combination, the *f* allele in the S subregion is associated with higher C3 levels than the *d* allele, and in the C3H series, the *d* and *k* alleles of S have higher C3 levels than the *b* allele. These findings agree with the effects of these alleles in the B10 series (Table 2). (2) C3 levels of five congenic and congenic recombinant strains of the B10 series, which have identical alleles in the IC and S subregions (*IC^d*, *S^d*),

were also compared. B10.A, B10.A(2R), B10.A(17R), B10.D2 and B10.A(5R) differ in the K, IA and IB subregions (*k*, *b*, *d* and *f* alleles) and also in the D subregion (*d*, *b* and *f* alleles). Their C3 levels, however (0.87 ± 0.12 , 0.89 ± 0.11 , 0.85 ± 0.09 , 0.86 ± 0.08 and 0.83 ± 0.12 , respectively), were not significantly different ($F_{136}^4 = 2.0$).

C3 levels in newborn mice are also influenced by gene(s) outside the *H-2* complex. Indeed, at 3 weeks of age significant differences ($P < 0.01$) were observed between C3 levels of mice with the same *H-2* but different backgrounds such as DBA/2J (0.54 ± 0.06) and B10.D2 (0.86 ± 0.08); B10.M (0.95 ± 0.08) and A.CA (0.81 ± 0.05); C3H.B10 (0.94 ± 0.2) and C57BL/10 (0.81 ± 0.09).

Because genetic polymorphism of human C3 can be detected by electrophoresis⁹ we subjected the plasma of mice with *H-2^k*, *H-2^d*, *H-2^b*, *H-2^s* and *H-2^f* haplotypes in different backgrounds (A, C57BL, C3H, DBA/2J and AKR/J) to high voltage electrophoresis in Agarose gel. No differences in the mobility of the C3 band were observed. We conclude that the levels of C3 in newborn mice are controlled by at least two genes, one of which is situated in the central region of the *H-2* complex, as shown by segregation analysis and by the studies on congenic and congenic recombinant mice. In adult mice, significant differences in C3 levels were no longer detectable. Perhaps these genes cease to function, or more likely, their influence is masked by the activity of other genes and/or environmental influences.

The nature of the *H-2* gene which controls C3 levels in ontogeny is unresolved. On the basis of preliminary mapping, it can be separated from the *Hom-1* gene¹⁰ situated at the K end of *H-2* (ref. 11) which influences testosterone levels and the relative weights of several organs which are hormone-regulated. Meruelo and Edidin¹² have demonstrated that basal levels of

Table 3 C3 levels in congenic mice with different S subregion of *H-2*

Congenic line	<i>H-2</i> subregions						C3 levels	F _y ^x	P
	K	IA	IB	IC	S	D			
A/SfSn	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	0.82	F ₆₂ ¹ = 32.1	< 0.001
A.AL	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	0.97		
C3H.B10	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	0.94	F ₆₁ ² = 6.5	< 0.005
C3H.OH*	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>k</i>	1.05		
C3H.OL*	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>k</i>	<i>k</i>	1.18		
B10.Br	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	0.96	F ₄₂ ¹ = 8.9	< 0.001
B10.M (17R)	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>f</i>	0.85		
A/WySn	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	0.74	F ₄₃ ¹ = 15.3	< 0.001
A.CA	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	0.81		

* The differences between C3 levels of C3H.OH and C3H.OL are significant ($F_{48}^1 = 4.21$; $0.05 > P > 0.01$).

cyclic AMP in the liver of adult mice segregate with *H-2* in F_2 mice, and also differ significantly among relevant combinations of congenic mice. They also present evidence to suggest that two genes, one at each end of the *H-2* complex, are involved. But the precise position of these genes within the subregions of *H-2* has not been determined. If one of the cyclic AMP genes is mapped within or close to the S subregion, the possibility of its direct or indirect involvement in the control of C levels should be considered.

This work was supported by grants from the National Institutes of Health.

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Received January 20; accepted February 18, 1976.

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Microtubule assembly mutant

WE report here a new maternal effect mutation (*nc*, for no cleavage) in the Mexican axolotl, *Ambystoma mexicanum*. This mutation involves a defect in the assembly of microtubules in the fertilised egg. There is a large literature describing the molecular and biochemical events which accompany the *in vitro* self-assembly of microtubules; the *nc* mutant is the first known system with a genetic lesion in the regulation of these events *in vivo*. This mutant will provide a means to examine directly the control of microtubule assembly, and may also be useful in the identification of the events which trigger initiation of cleavage after activation of the egg at fertilisation.

Eggs spawned by female axolotls homozygous for *nc* seem to be morphologically normal. They show normal interaction with sperm, which enter and cause activation of the eggs as determined by the standard criteria of cortical changes, formation of polar bodies and swelling and migration of the pronuclei. But no mitotic apparatus or cytasters are assembled and no cleavage occurs. The mutant eggs do contain a pool of soluble tubulin indistinguishable from that in normal eggs. (A detailed description of the characterisation of axolotl tubulin will be published elsewhere.)

We have found that the lesion in the *nc* mutant eggs can be partially corrected by injection of microtubule fragments. If fertilised *nc* eggs (which are arrested after the cortical activation stage) are injected with fragments of the outer-nine doublet tubules prepared from sea urchin sperm tail axonemes, they begin to cleave and proceed to partial blastula as shown in Fig. 1. Cleavage begins at about the time it would have started had the eggs initiated cleavage normally. Normal fertile axolotl eggs cleave about 7 h after spawning at 18 °C; fertilised *nc* eggs injected with doublet tubule fragments 1 or 2 h after spawning begin to cleave 5–6 h later. The initial cleavages are fairly normal in appearance in the animal hemisphere but there is little cleavage in the vegetal region of the eggs. Later cleavages become more irregular, resulting in the formation of partial blastulae, and the eggs finally arrest. The doublet microtubule fragments which elicit cleavage are composed of tubulin; dynein and other high molecular weight proteins are absent (Fig. 2).

No cleavage results after injection of soluble tubulin, buffer, non-tubulin particles or normal germinal vesicle nucleoplasm. In addition, no cleavage results after injection of doublet tubule fragments into activated *nc* eggs followed by bathing the eggs in colchicine at a concentration which arrests cell division of normal fertile axolotl eggs. Activation of the egg is necessary for the correction effect: injection of microtubule fragments into unactivated infertile eggs from either *nc* mutants or normal females does not cause cleavage.

In some preliminary experiments, eggs were injected with basal bodies isolated from *Chlamydomonas reinhardtii*. These preparations are much more heterogeneous than the doublet tubule fragments and the results were more variable and difficult to assess. A very small proportion of eggs injected with basal bodies underwent cleavage comparable with that caused by doublet tubules; most did not cleave at all, and some underwent abnormal cleavage or fragmentation, which is clearly distinguishable from cleavage.

The *nc* mutant eggs can be phenocopied by electrically stimulating infertile eggs from normal females. This causes

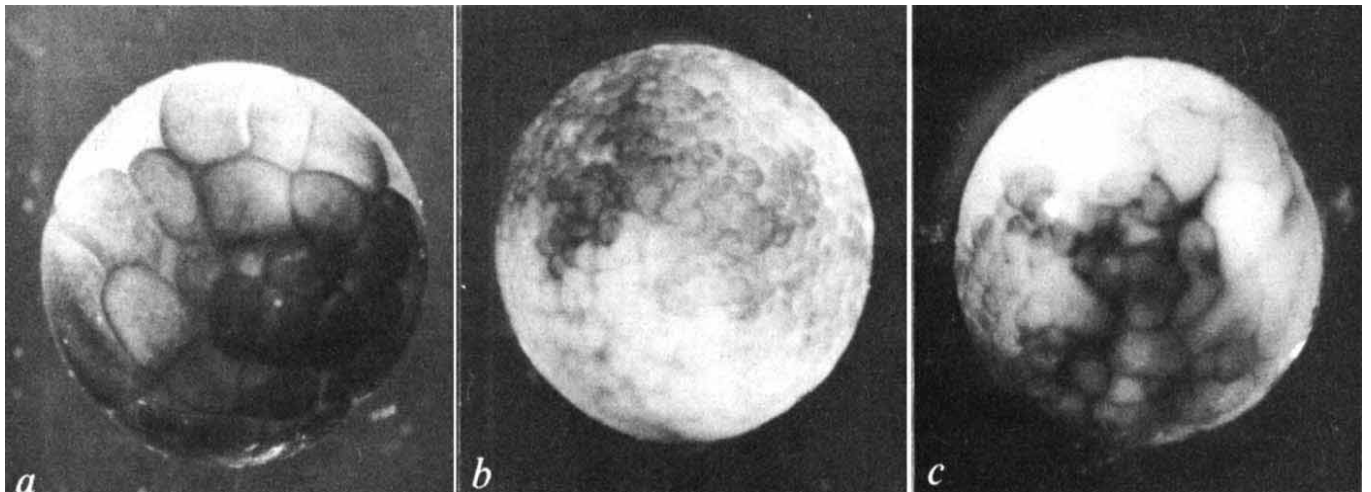


Fig. 1 Cleavage of normal and corrected *nc* mutant eggs. *a*, Normal fertilised egg in mid-cleavage. *b*, Mutant egg corrected by injection of doublet tubules. Cleavage has progressed in the animal hemisphere to form a partial blastula similar in appearance to a normal blastula. This is an example of the best correction observed. *c*, Mutant egg corrected by injection of doublet tubules. This is an example of the poorest correction observed containing patches of irregular sized cells.

Table 1 Induction of cleavage in *nc* mutant and normal axolotl eggs

Treatment	Mutant: activated <i>nc</i> eggs	Phenocopy: electrically activated normal eggs	Unactivated eggs: infertile <i>nc</i> or normal eggs
Injection of doublet tubule fragments prepared from sea urchin sperm tails	+*†	+	—
Injection of buffer	—*†	—	—
Injection of soluble tubulin prepared from <i>Drosophila</i> embryos	—*†	—	—
Injection of germinal vesicle nucleoplasm from normal eggs	—*	—	—
Injection of heat-killed bacteria (<i>Staphylococcus aureus</i>)	—*	—	—
Injection of doublet tubule fragments followed by bathing in 10^{-3} M colchicine‡ or 10^{-4} M vinblastine	—†	—	—

+, Initiation of cleavage; —, No cleavage.

* Activated by interaction with sperm (that is, fertilised eggs).

† Activated by electrical stimulation (of infertile eggs).

‡ This concentration of colchicine causes arrest of normally dividing fertile embryos. The relatively high concentration is necessary because of the impermeability of the eggs.

activation of the eggs so that morphologically they appear the same as fertilised eggs but they do not subsequently undergo cleavage. (Occasionally some of the electrically activated eggs fragment; these eggs are not used in correction experiments.) Electrically activated eggs also initiate cleavage to form partial blastulae when injected with doublet tubule fragments. Likewise, electrically activated infertile *nc* mutant eggs initiate cleavage when injected with doublet tubule fragments. A summary of the results of microinjection experiments is given in Table 1.

Preliminary light and electron microscopic observations of sections of the partial blastulae resulting from injected *nc* eggs show that the blastomeres are surrounded by complete cleavage furrows and that each blastomere contains an area in which the cytoplasm is cleared and in which parallel arrays of microtubules are formed. These arrays are occasionally but not usually

organised into a shape resembling a true spindle. Chromosome fragments are found associated with these arrays in some (but not all) of the blastomeres. The initiation of cleavage thus appears to be due to a triggering of microtubule assembly from the existing pool in the egg, since the amount of tubulin contained in the injected fragments is less than 10% of the amount already present in the egg. In addition, in some cross sections we have observed doublet tubules, indicating that the injected fragments retain their morphological integrity.

It is our hypothesis that the lesion in the *nc* mutant lies in an early microtubule assembly event stimulated by activation, an event possibly analogous to the formation of mitotic organising centres in activated (but not in unactivated) eggs of the surf clam *Spisula*, as described by Weisenberg and Rosenfeld¹. Although the correction obtained is not complete, in that the eggs do not finish embryonic development, nevertheless the normal timing of cleavage initiation and the presence of microtubule arrays and chromosome fragments in the blastomeres indicates that a process resembling normal cell division is elicited. One very intriguing aspect of the *nc* eggs is that during activation they seem to complete meiosis. We do not know whether this indicates a molecular difference in the meiotic and mitotic spindles or a difference in regulation.

Fraser achieved parthenogenetic development of *Rana pipiens* eggs by injection of crude fractions possibly containing microtubules². Injection of axonemes from *Tetrahymena* or *Chlamydomonas* did not elicit cleavage; Heidemann and Kirschner³ postulated that Fraser's active fractions contained centrioles. Ohta and Iwamatsu^{4,5} achieved partial development of infertile eggs of the medaka by injecting them with flagella or microtubules prepared from sea urchin sperm. In both these organisms, unlike the axolotl, parthenogenetic development of infertile eggs is fairly readily induced and activation of the egg occurs during microinjection. Heidemann and Kirschner³ demonstrated that injection of isolated basal bodies into infertile eggs of *Xenopus laevis* elicited both aster formation and initiation of a type of cleavage with irregular furrows and fragmentary blastomeres. This is similar to what we have scored in our experiments as fragmentation. These authors do not discuss the state of activation of the *Xenopus* eggs after injection of basal bodies. They found, however, that isolated flagella or axonemes were ineffective in inducing aster formation or cleavage when injected into infertile *Xenopus* eggs.

In the *nc* axolotl mutant there is a clear-cut separation between the events of activation and subsequent initiation of cell division. In this mutation, for the first time a genetic lesion has been found which involves the role of microtubule assembly in the initiation of cell division and subsequent embryonic development. A detailed description of this mutant will be published elsewhere.

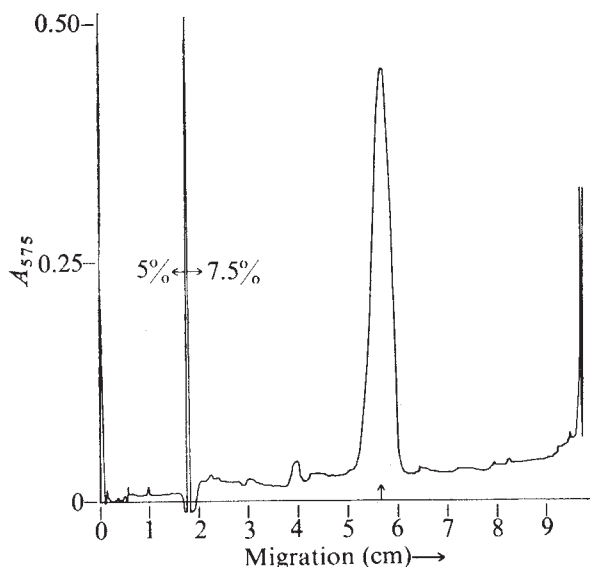


Fig. 2 Purity of injected doublet microtubule fragments. Doublet tubules were solubilised in 8 M urea–0.2% sodium dodecyl sulphate (SDS) buffer and electrophoresed on an SDS–urea polyacrylamide gel which was then stained with Coomassie blue and scanned. The running gel consisted of a 2-cm gel containing 5% acrylamide poured on top of an 8-cm gel containing 7.5% acrylamide. A stacking gel was poured over this. This allows good resolution of both high and low molecular weight sperm tail proteins. Tubulin (indicated by arrow) accounts for 97% of the protein present in the doublet tubule fragments used in our experiments. Dynein, which would be represented by a peak in the 5% gel, is absent from this preparation but is clearly visible in gels of whole axonemes.

We thank J. W. Brandis and J. E. Loyd for doublet tubules, L. H. Green for soluble tubulin, F. R. Turner for electron microscopy and L. Lawrence for photography. This work was supported by the US National Science Foundation.

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Received December 8, 1975; accepted February 12, 1976.

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Mechanical properties of vascular smooth muscle cells *in situ*

ALTHOUGH the histology and ultrastructure of smooth muscle cells have been studied extensively there is little direct evidence concerning their mechanical properties. Their small size^{1,2} and lack of tendon connections have prevented them from being mounted on a myograph, as has been done with striated muscle fibres³. Moreover, their multiplicity and optical properties make it difficult to visualise them in whole smooth muscle preparations. Although isolated smooth muscle cells^{4–6} can be seen to contract

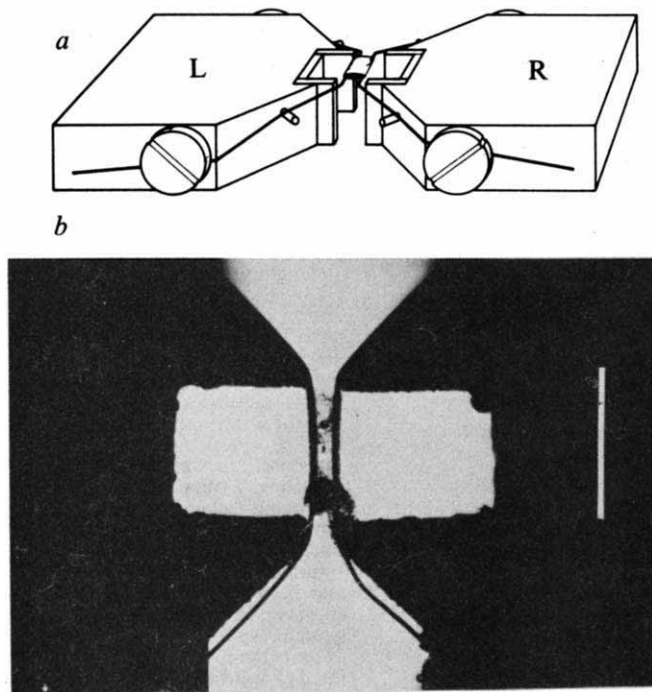


Fig. 1 *a*, Sketch showing method of mounting vessel segment. The vessels were excised from the animal together with about 1 cm of the proximal arterial tree. The latter was threaded on to a 32- μ m tungsten wire and used to guide the wire into the lumen of the test vessel. The unwanted tissue was then dissected away and the wire was clamped at each end under tension to the left-hand support (L). A second wire was then passed through the lumen and similarly attached to the right-hand support (R). The supports were mounted respectively on a tension transducer (DSC 6, Kistler-Morse, resonant frequency 400 Hz with support mounted) and a piezoelectric vibrator (PZ40, Burleigh, range 19 μ m, frequency response 0–2 kHz). The vibrator was in turn mounted on a micrometer translator for gross movement. Movement of R was detected using an eddy-current displacement transducer (KD 2300.5 SU Kaman). The supports were immersed in a chamber through which solutions were circulated. *b*, Low power micrograph of a vessel when mounted as described in (*a*) and viewed from above. The bar shows 1 mm.

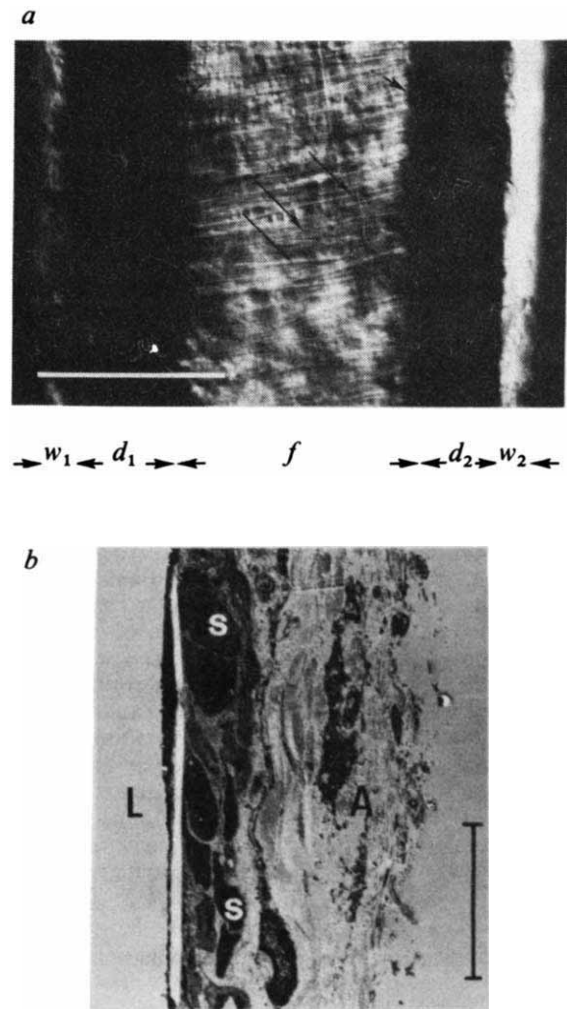


Fig. 2 *a*, High power micrograph of the vessel shown in Fig. 1*b* with focal plane in the media of the upper wall. The general outline of the smooth muscle cells is seen (the long arrows show a number of cell boundaries). The two dark areas (short arrows) are the 32- μ m mounting wires. Photographed using Nomarski interference contrast optics⁷ (Zeiss Universal) with $\times 40$ water immersion objective. Condenser aperture fully opened to reduce depth of field to about 4 μ m. The symbols indicate the dimensions which were measured using an ocular micrometer. The internal circumference, L , was calculated from the equation $L = (d_1 + d_2) [1 + (\pi/2)] + 2f$. For this vessel $L_0 = 361$ μ m and the segment length was 665 μ m. The bar shows 50 μ m. *b*, Electron micrograph of vessel shown in (*a*) sectioned radially to show the smooth muscle cells (S) and adventitia (A) in cross section. L indicates the lumen. The vessel was fixed at 36 °C at conclusion of the experiment after equilibration in relaxing solution. The vessel was prefixed on the myograph in 2.5% glutaraldehyde, 75 mM cacodylate, 4.5% sucrose, then removed from myograph and postfixed in OsO₄, 50 mM cacodylate, then blockstained in saturated aqueous uranyl acetate and embedded in Epon¹⁹. The bar shows 10 μ m.

when stimulated, it has not been possible to measure the forces they develop. There are, therefore, still serious doubts about the extent to which the mechanical properties of whole smooth muscle preparations are an accurate reflection of the properties of smooth muscle cells. We have therefore developed a technique to obtain mechanical data from a smooth muscle preparation which only contains about 1,000 smooth muscle cells, and which is thin enough for the cells to be visualised by Nomarski interference microscopy⁷. This technique provides for the first time the means to study the mechanical properties of smooth muscle cells *in situ*.

We used segments, about 0.7 mm long, of small (100 μ m lumen) arterial resistance vessels. They were taken from the mesenteric bed of 5-month-old Wistar Kyoto rats from a point immediately proximal to the intestinal wall. Each vessel segment

was threaded on to two wires attached respectively to a force transducer and a displacement device⁸ (Fig. 1). This arrangement enabled the wall tension P and vessel internal circumference L to be controlled. The myograph was mounted on the stage of a microscope, enabling us to measure vessel dimensions (Fig. 2a). Oxygenated physiological salt solutions were circulated through the test chamber at 36 °C, pH 7.4. The vessels were maximally activated by K^+ -depolarisation in the presence of 5 mM Ca^{2+} (ref. 9) and relaxed using the same solution with Ca^{2+} replaced by 1 mM EGTA. At other times the vessels were held in Ringer solution¹⁰. The overall myograph compliance was less than $0.5 \mu\text{m mN}^{-1}$ and has not been corrected for. We report here results obtained from four typical experiments. The vessels were all held at an internal circumference, L_0 , for which their active wall tension was a maximum P_0 . Results are presented as mean $\pm$ s.e. The figures all refer to the data obtained from one of these vessels.

Figure 2a shows the appearance of the vessels in the light microscope in the conditions described in the legend. The general outline of the smooth muscle cells was clearly seen and the dimensions of individual cells were measured. The cells were spindle shaped, of length about 65 μm and diameter 4 μm , and were orientated approximately perpendicular to the wires. In some cells internal structures such as the nuclei and mitochondria could also be identified. When the vessels were activated, changes in the appearance of the cells were followed and although there was some rearrangement of the cells there was little change in their average length. The dynamic behaviour of the smooth muscle cells was then examined by monitoring the microscope image with a television camera and recording it on videotape. This was done during quick (5 ms) changes in internal circumference imposed by the displacement device while the vessels were activated. By replaying the tape one

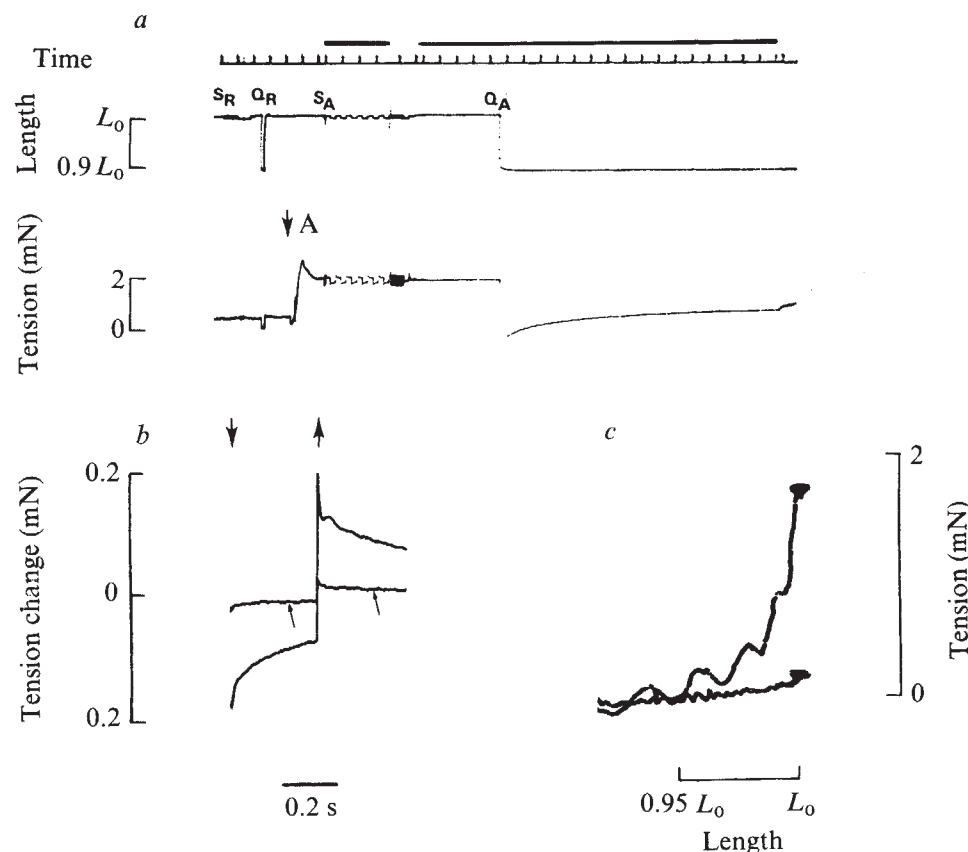
frame at a time, the frames immediately before and after the length change (which were separated by 17 ms) could be compared. Comparisons of the two frames showed that for length changes up to at least 10% L_0 , the length of any smooth muscle cell was always a fixed proportion of the vessel internal circumference. In other words, changes in the strain of a vessel produced the same changes in the strain of cells. Thus active force-strain data obtained from these vessels were a measure of the active characteristics of the individual smooth muscle cells.

At the conclusion of the experiments the vessels were fixed on the myograph at L_0 . Electron micrographs of longitudinal sections confirmed that the structures identified in Fig. 2a were indeed smooth muscle cells, and that their contractile material was generally well organised and orientated parallel to the long axis of the cells. Transverse sections (Fig. 2b) showed that the smooth muscle cells were closely packed in one to three layers, taking up 67% of the medial layer and 20% of the total wall.

Typical mechanical data obtained from the vessels are shown in Fig. 3. The total force developed by the vessels corresponded on the basis of the light and electron microscopy to an active wall stress of $72 \pm 13 \text{ mN mm}^{-2}$, giving an active force per smooth muscle cell of about 5 μN and an active stress for the smooth muscle cells of 360 mN mm^{-2} . The last value can be compared with the figure of 370 mN mm^{-2} obtained for smooth muscle cells in hog carotid artery¹⁰ and at least 300 mN mm^{-2} for frog striated fibres¹¹. The dynamic stiffness was measured in two ways: from the step response of the vessel (Fig. 3b), and from the initial portion of the response of the vessels to a quick release sufficient to discharge the tension (Fig. 3c). These showed that the dynamic stiffness of small displacements was $67 \pm 6 P_0/L_0$ and $81 \pm 16 P_0/L_0$, respectively. The complete instantaneous elastic characteristic (Fig. 3c) was fitted approximately by an

Fig. 3 Records of mechanical responses obtained from vessel shown in Figs. 1 and 2. a, Oscillograph record. The upper trace shows time; markers at 1-min or, under the bars (where the chart

speed was increased), 1-s intervals. The centre trace shows length. The lower trace shows tension. The vessel was initially in relaxing solution (see text). At $\downarrow A$ the solution was changed to activating solution. The response of the vessel to small (0.0028 L_0), repetitive (1.57 Hz) step changes in length was measured at S_R and S_A . The mean responses are shown in (b). The response of the vessel to quick releases (during 10 ms) through 0.086 L_0 was measured at Q_R and Q_A . The length-tension relationships during these releases are shown in (c). b, Mean of 32 tension responses to release ($\downarrow$) and stretch ($\uparrow$), obtained using signal averaging techniques sampling at 5-ms intervals, measured at the times indicated in (a) in relaxing solution (centre curves, arrowed) and in activating solution (outer curves). The active release and stretch responses, when expressed as unit step responses, $u_{-1}(t)$, are fitted approximately by the equations $u_{-1}(t) = -(P_0/L_0) (57.3 - 14.5 \log t)$ and $u_{-1}(t) = (P_0/L_0) (60.1 - 15.7 \log t)$, respectively. c, Oscilloscope plots of the length-tension relationship during the quick releases shown in (a) in relaxing solution (lower curve) and activating solution (upper curve). Vertical deflection represents tension and horizontal deflection represents length²⁰. The difference between the active and relaxed responses is fitted by the equation $P = P_0 \exp[-77.6 (\Delta L/L_0)]$. The oscillations on the upper curve are mechanical artefacts.



exponential equation as in hog carotid artery¹². Analysis of the step responses (Fig. 3b) show that the rate constants of the tension decay and recovery (if defined as $(dP/dt)/(P_0 - P)$) decreased throughout decay and recovery, but that their initial values were at least $0.038 \pm 0.005 \text{ ms}^{-1}$. The corresponding figure for frog striated fibres at 0°C is 0.4 ms^{-1} (ref. 13).

For the reasons discussed above, the data presented here represent the characteristics of smooth muscle cells *in situ* and, we believe, provide a basis for a better understanding of the relationship between structure and function in the smooth muscle cell. Structural studies^{14,15} have shown that the contractile apparatus of smooth muscle cells is a sliding filament mechanism but that it is much less regularly organised than that of striated muscle. It is not known, however, how the contractile filaments transmit the force they generate to the extracellular environment. This contrasts with the situation for skeletal fibres, where it is clear that the contractile filaments are arranged in myofibrils which run the entire length of the fibre, being attached to the tendons at each end¹⁶. The dynamic stiffness of these fibres ($195 P_0/L_0$) and their instantaneous elastic characteristic (IEC), which is approximately linear, are thought to be primarily the property of the crossbridges between the contractile filaments^{17,18}. Our data for smooth muscle cells show them to have about half the dynamic stiffness of skeletal muscle fibres, while their IEC does not seem to be linear. This suggests that their dynamic compliance is divided between crossbridge compliance and a nonlinear compliance in intracellular supporting structures.

Previous mechanical studies on smooth muscle have used much larger preparations and the dynamic stiffnesses reported have been rather lower. The highest dynamic stiffness so far reported has been in hog carotid artery ($58 P_0/L_0$)¹². It seems likely that the differences between these results and ours may be due to a greater amount of connective tissue between the smooth muscle cells in the larger preparations. As we have shown above, in our preparation the effect of such intercellular compliance is minimal. We believe therefore that the preparation we have described may be useful in the detailed examination of the intrinsic mechanical properties of smooth muscle cells.

We thank Mr W. G. Boldosser for taking the electron micrographs and Mr Joseph P. Trono for constructing the myograph. The tungsten wire used for mounting the vessels was given by Westinghouse Corporation, Lamp Division. The work has been funded by the US National Institutes of Health.

M.J.M. is on leave-of-absence from the Biophysics Institute, Aarhus University, Aarhus, Denmark.

Note added in proof: We should like to draw attention to a recent report²¹ of direct measurements of the force developed by isolated smooth muscle cells.

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Received December 15, 1975; accepted February 17, 1976.

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Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle

RESTING frog skeletal muscle, when stretched at a constant velocity, exhibits a force response which, for length changes less than about 0.2%, is elastic in nature. First observed by Hill¹, this response was attributed to a short range elastic component (SREC) that was thought to be a property of a small number of cross bridges which, even in the resting state, extend from the myosin-containing thick filaments to interact with sites on the actin-containing thin filaments. This view was criticised² on the grounds that the SREC stiffness remained approximately constant as the amount of thick and thin filament overlap was decreased by increasing muscle length. On the basis of the sliding filament theory^{3,4}, SREC stiffness would be expected to decrease because reduced amounts of thick and thin filament overlap would result in a decreased number of cross bridges available to interact with thin filament sites. Flitney and Hirst⁵ have reported that in twitch contractions of frog skeletal muscle a short range elastic response is present and is similar in form to that of resting muscle. In this case, however, a directly proportional relationship was found between the short range stiffness and the amount of overlap between the thick and thin filaments, which suggests that this stiffness is a property of attached cross bridges. To investigate the possible presence of attached cross bridges in resting muscle, we have measured the calcium dependence of the short range stiffness in skeletal and cardiac muscle preparations in which the myofibrils were directly exposed to the bathing medium.

Single muscle fibres were isolated from the semitendinosus muscle of the frog (*R. pipiens*) and their sarcolemmata were

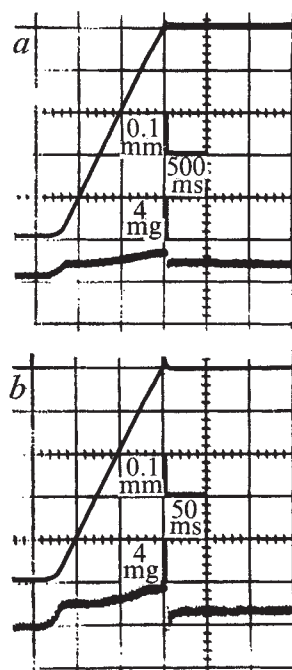


Fig. 1 *a*, Force response ($E = 2.93 \text{ kg cm}^{-2}$; lower) of living frog skeletal fibre at rest to constant velocity (0.06 ML per s ; total length change, $\Delta L = 7.46\%$ of initial length, L_i) stretch (upper). $SL = 2.2 \mu\text{m}$; temperature, 4°C . *b*, As for *a* except velocity is 0.60 ML per s and $E = 5.70 \text{ kg cm}^{-2}$. Responses are typical, demonstrating a steep initial rise which is the elastic part of the response, reaching an elastic limit at a change in length of about 0.3% and followed by Hill's¹ frictional phase as the stretch is continued. Short range elastic stiffness (expressed as modulus, E) is calculated as the height of the initial, fast part of the force response divided by the corresponding length change.

either removed by mechanical skinning⁵ or made highly permeable by chemical treatment (that is, chemical skinning) with glycerine and a non-ionic detergent⁶. Thin longitudinal strips were cut from rabbit right ventricular papillary muscles and placed in glycerinating solution⁶ at 0–5 °C for 3 d. Muscle segments, 1–3 mm long, were attached to wires extending from a force transducer and servo motor by means of glass jaws—two pieces of glass coverslip held flat together by slightly sprung strips cut from a stainless steel razor blade. This connection proved to be isometric, since no muscle movement with respect to the jaws was observed through the light microscope at $\times 400$ magnification. Constant velocity length increases were applied at one end of the muscle preparation through an arm attached to the servo motor. Force was measured using a capacitance transducer with a sensitivity of 1 mV mg^{-1} and a natural resonance frequency of 800 Hz. All measurements were made at 4 °C. The solutions, apparatus and methods for controlling free Ca ion concentration using EGTA have been described⁶. Sarcomere length (SL) was measured and monitored continuously using a light microscope⁶.

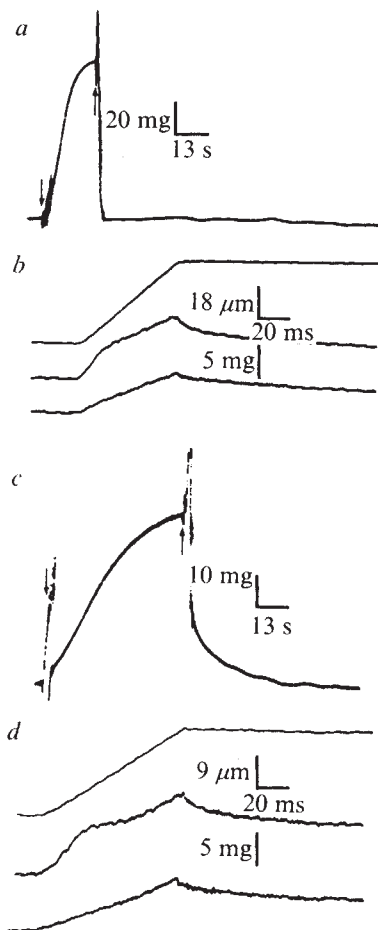


Fig. 2 *a*, Contraction time-course of glycerinated skeletal fibre when activating solution is applied ($p\text{Ca} = 5.76$; first arrow) followed by relaxing solution ($p\text{Ca}$ about 9; second arrow). SL = 2.28 μm . *b*, Force responses of glycerinated skeletal fibre to constant velocity (0.34 ML per s; total $\Delta L = 1.90\%$ L_i) stretch (upper) in relaxing solution ($p\text{Ca}$ about 9; $E = 4.55 \text{ kg cm}^{-2}$; lower) and activating solution ($p\text{Ca} = 6.51$; $E = 15.3 \text{ kg cm}^{-2}$; middle). SL = 2.28 μm . *c*, Contraction time course of glycerinated papillary muscle strip when activating solution is applied ($p\text{Ca} = 5.76$; first arrow) followed by relaxing solution ($p\text{Ca}$ about 9; second arrow). SL = 2.14 μm . The slower time course relative to that of the skeletal fibres is typical of the heart preparations used. *d*, Force responses of glycerinated papillary muscle strip to constant velocity (0.46 ML per s; total $\Delta L = 3.66\%$ L_i) stretch (upper) in relaxing solution ($p\text{Ca}$ about 9; $E = 2.25 \text{ kg cm}^{-2}$; lower) and activating solution ($p\text{Ca} = 6.51$; $E = 6.75 \text{ kg cm}^{-2}$; middle). SL = 2.14 μm .

The calcium sensitivity of the three preparations was quantified to establish a basis for comparison of the stiffness measurements which follow. In each case, the calcium threshold for active tension development was near a $p\text{Ca}$ (negative log of free Ca ion concentration) of 6.9. At higher calcium ion concentrations the typical S-shaped relationship between tension and $p\text{Ca}$ was confirmed⁶; in all cases no further increases in active tension development were noted at $p\text{Ca}$ less than 6.

Short range elastic stiffness was measured first on living, resting single frog skeletal muscle fibres (Fig. 1). At a velocity of stretch of 0.06 muscle lengths (ML) per second, an elastic modulus (E) of 2.93 kg cm^{-2} was measured, comparing well with the value of 2.23 kg cm^{-2} found by Lannergren⁷ using a slower rate of stretch (about 0.005 ML per s) and a similar preparation. In accordance with the findings of Hill¹, we found that the measured value of E rose as a function of increasing velocity of stretch, an effect especially prominent at velocities greater than 0.004 ML per s. A stretch rate of around 0.40 ML per s was chosen for use in the stiffness measurements involving the chemically activated preparations in order to take advantage of the enhancement of the response at higher stretch velocities.

Constant velocity stretches were applied to the mechanically and chemically skinned muscle preparation (Fig. 2) while in relaxing solution, in which there is no added caffeine. The nearly exact similarity of the tension responses of the type reported by Hill for living muscle, and shown in Fig. 1, was found. Instead, tension increased steadily with increasing length to a level characteristic of the final length, there being some slight stress-relaxation when movement stopped. The form of the response changed considerably, to that typical of living muscle, when solutions containing small amounts of added calcium were applied. The threshold for this transformation was about $p\text{Ca}$ 7. At $p\text{Ca}$ less than 7, the measured short range stiffness increased sharply with decreasing $p\text{Ca}$. No stiffness measurements were made, however, at $p\text{Ca}$ less than 6.09, because marked decreases in active tension development during subsequent contractions were observed after stretches at these $p\text{Ca}$ levels.

We investigated the possibility that the sarcoplasmic reticulum (SR) has a role in determining the tension response of muscle to constant velocity stretch. Caffeine (10 mM) in the presence of 0.1 mM EGTA was applied to each preparation after successive treatments with a normal activating solution ($p\text{Ca}$ 6.7) and relaxing solution containing low EGTA (0.1 mM), a procedure similar to that described by Endo⁸. Only the mechanically skinned skeletal fibres showed a functionally intact SR, as judged by a large tension response of transient duration after application of caffeine. The nearly exact similarity of the tension responses to stretch in the mechanically and chemically skinned fibres indicates that the SR does not have a major influence in the determination of short range stiffness.

These results show that the short range stiffness of resting muscle depends on the concentration of calcium in the fluid bathing the myofibrils. Calcium is believed to trigger the interaction of cross bridges with sites on the thin filaments by binding with the regulatory protein troponin which is located at intervals along the thin filaments⁹. Also, X-ray diffraction data suggest that in skeletal muscle activation may affect cross-bridge configuration causing the cross bridges to move out from the thick filaments and toward the thin filaments¹⁰. Other X-ray work¹¹ indicates that in living, isolated heart muscle a few cross bridges may be in an activated, though not necessarily attached, position while the muscle is at rest. Therefore, the short range stiffness seems to be a property of calcium-activated cross bridges which have either become attached to thin filament sites or at least changed from a resting to an activated position. To this extent, our work confirms Hill's hypothesis¹ that cross bridges are the source of the SREC.

The finding that the short range elastic response is virtually absent in muscles bathed in relaxing solution but is present at pCa near 7 implies that the calcium concentration in the myoplasm of living fibres at rest is about pCa 7. Finally, the short range elasticity has for the first time been shown to be present in a heart muscle preparation. The discovery of this elastic response as a characteristic of both heart and skeletal muscles suggests that the calcium mechanism which underlies the response is the same in the two cases.

This work was supported by grants from the National Institutes of Health, the US National Science Foundation, the Muscular Dystrophy Association of America, the American Heart Association and the Massachusetts Heart Association, and by a National Institutes of Health post-doctoral fellowship to R.L.M.

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Ultrastructural identification of granules containing oxytocin and vasopressin

OXYTOCIN and vasopressin, the hormones of the mammalian hypothalamo-neurohypophyseal complex, are synthesised in the supraoptic (SON) and paraventricular (PVN) nuclei and are secreted into the blood stream from the neurohypophysis. It is now thought that the two hormones are produced in both nuclei, but are stored, together with their corresponding neurophysins, in distinct neurosecretory granules located in separate perikarya and axons, on the basis of data from various species including fish, reptiles, birds and mammals¹⁻⁴. This study describes cytochemical experiments carried out on tissue from normal rats and from rats homozygous for hereditary diabetes insipidus (Brattleboro strain) that support this view and allow a tentative identification of the two granule types.

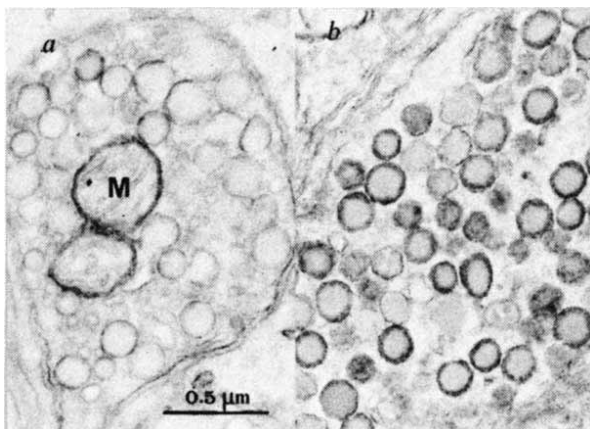


Fig. 1 Secretory axons in the neurohypophysis of normal rats. Potassium ferrocyanide-OsO₄ method. *a*, A neurosecretory axon with non-reactive granules. *b*, An axon in which the granules have the ring-shaped electron-dense deposit around their core. M = mitochondria.

Three methods believed to detect glycoproteins at the ultrastructural level were used: the periodic acid-thiocarbohydrazide-silver proteinate technique⁵, the periodic acid-chromic acid-silver methenamine technique⁶ and the potassium ferrocyanide-osmium technique⁷. Following each reaction, two distinct types of neurosecretory axon were recognised within the neural lobes of normal rats (Sprague-Dawley strain)^{8,9}. In one type of axon, most granules had a ring-shaped silver or ferrocyanide-osmium deposit around their core. In the other type of axon, all granules had an homogeneous content of low electron density (Fig. 1). This contrasting aspect could be enhanced further by combining uranyl acetate impregnation *en bloc* with a subsequent proteinate silver reaction on ultrathin sections. Figure 2 shows the characteristic, positive reaction of the granules in one axon from a neural lobe treated with uranyl acetate *en bloc*. Variations in the plane of section may account for the unequal intensity of the reaction in granules of the positive axon. A small number of unreactive granules were sometimes seen intermingled among reactive granules, but they usually had an electron-lucent space between the membrane and core which was absent in the granules seen in

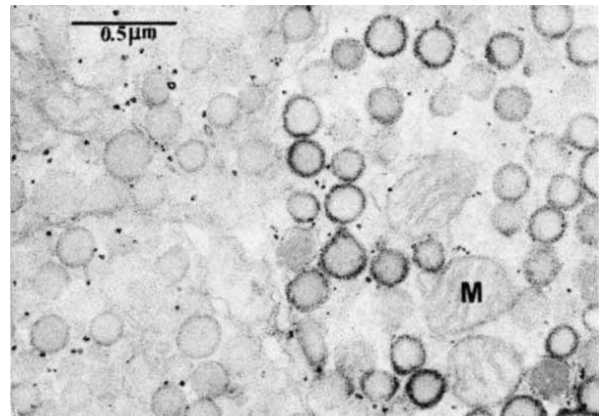


Fig. 2 Secretory axons in the neural lobe of a normal rat. Uranyl acetate and periodic acid-thiocarbohydrazide-silver proteinate method. Note presence of axons containing non-reactive granules (left), and reactive granules in another, adjacent axon (right).

non-reactive axons. Of 3,400 axons in normal Sprague-Dawley neurohypophyses, 68% were found to be positive. Axons of each type frequently occurred in small groups.

The same methods revealed a similar duality in the hypothalamic magnocellular nuclei. This was particularly obvious in PVN, where perikarya and axons contained either reactive granules or non-reactive ones. In SON, the non-reactive type seemed to be less frequent, at least in the portion lateral to the optic chiasma, the region usually investigated for technical convenience.

These results indicate, therefore, that a distinction can be made between two types of neurosecretory axons and perikarya in normal rats. Since homozygous Brattleboro rats are characterised by an absolute deficiency in vasopressin (and vasopressin-neurophysin) biosynthesis^{10,11}, the same methods were applied to 19 of these animals to identify those axons and perikarya containing oxytocin granules. Before fixation, 9 animals were treated for 1 month with exogenous vasopressin (Pitressin tannate, Parke-Davis, 1 IU d⁻¹ subcutaneously)¹². We found that granulated axons were more frequent in the neurohypophysis of treated rats. They generally occurred in clusters surrounded by areas devoid of neurosecretory granules. All the granules observed in the neural lobes of homozygous Brattleboro rats were non-reactive (Fig. 3*a*). There was some deposit of silver proteinate on the granule-limiting membranes, in particular following treatment with uranyl

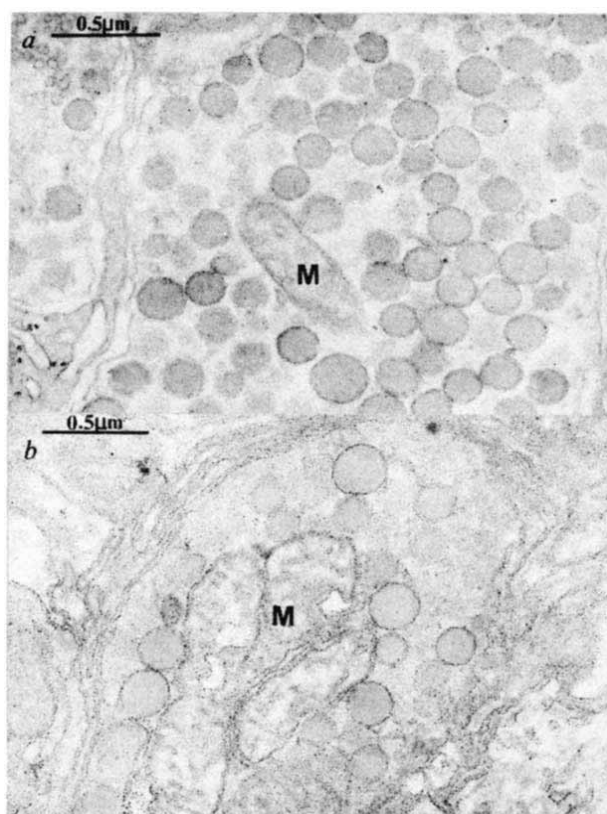


Fig. 3 Micrographs from homozygous Brattleboro rats treated with Pitressin tannate. Periodic acid-thiocarbohydrazide-silver proteinate method. Note that in the neurohypophysis (a) and in the hypothalamic paraventricular area (b) secretory axons contain only axons of the non-reactive type.

acetate *en bloc*, but the granule cores appeared homogeneous and did not show the characteristic annulate density. As in normal rats, we also studied the PVN and that part of the SON which is adjacent to the optic chiasma. The granules found in the axons and perikarya of these nuclei in homozygous Brattleboro rats were also non-reactive (Fig. 3b). Normal rats of the same strain (Long-Evans) served as controls. Of 1,600 neurohypophysial axons examined, 34% contained the reactive granules.

In view of the absence of reactive granules in homozygous Brattleboro rats, we propose that the reactivity of the neurosecretory material to the histochemical 'stains' used in this study is associated with the vasopressin-containing granules. Oxytocin is thus stored in the non-reactive granules. We do not at present know what accounts for the reactivity of the vasopressin granules, but their difference from the oxytocin granules may be in glycoprotein composition. Our results also support the idea that vasopressin and oxytocin are present in distinct neurones found in both PVN and SON.

We thank Mrs S. Rua for technical assistance, the Institut für medizinischbiologische Forschung, Fillensdorf, for supplying the Brattleboro rats, and the CNRS and INSERM for support.

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Primary structure of human β -lipotropin

AFTER the discovery and isolation of β -lipotropin from sheep pituitary glands^{1,2}, the hormone has also been obtained in highly purified form from bovine³, porcine⁴⁻⁶, and human^{7,8} pituitaries. The complete amino acid sequences of ovine^{9,10} and porcine¹¹⁻¹³ hormones have been proposed. To gain insight into the evolutionary history of lipotropins and their structure-function relationships, and in view of recent data on the opiate agonist activity of peptide fragments from β -lipotropin^{14,15}, we report here the complete amino acid sequence of the human hormone.

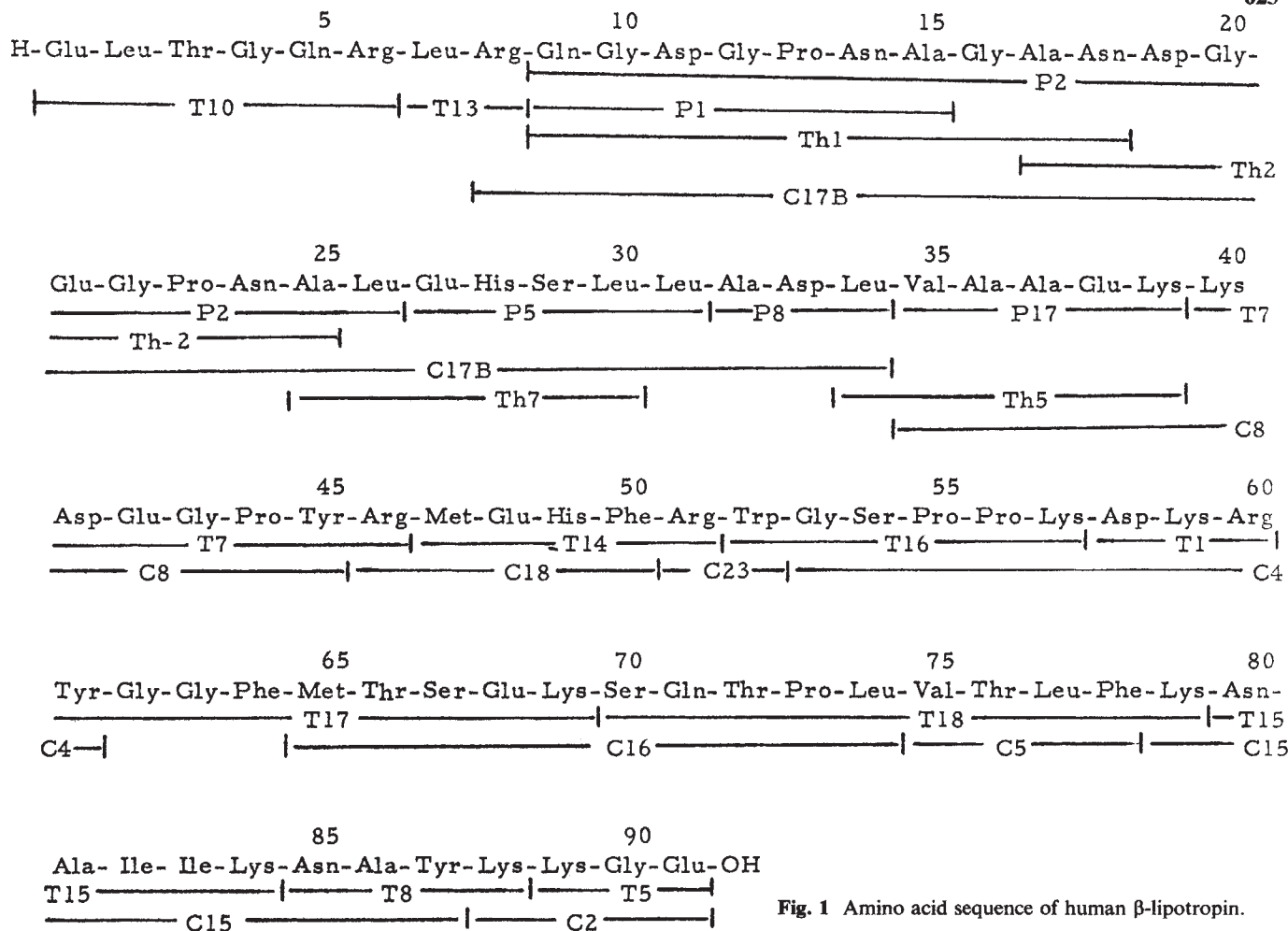
Highly purified β -lipotropin (β -LPH) was obtained from fresh-frozen human glands by the procedure previously described⁹ for the ovine hormone with minor modifications. It behaved as a single component in disc electrophoresis and partition chromatography experiments. Sedimentation equilibrium data gave a molecular weight of 11,700. Amino acid analysis, performed in the automatic amino acid analyser by the procedure of Moore¹⁶ gave the following empirical formula (91 amino acid residues): Trp (1) Lys (9) His (2) Arg (5) Asp (10) Thr (4) Ser (4) Glu (11) Pro (6) Gly (11) Ala (8) Val (2) Met (2) Ile (2) Leu (8) Tyr (3) Phe (3). The NH₂-terminal sequence as determined by the dansyl-Edman procedure^{17,18} was found to be: Glx-Leu-Thr-Gly-Glx-Arg-Leu-Arg-.

For the primary structure determination, a tryptic digest of β -LPH (3 mg) was fractionated by the two-dimensional paper chromatography-electrophoresis as described previously¹⁹. The eluates from each spot were submitted to amino acid¹⁶ and sequence^{17,18} analyses. Amino acid contents of the twelve tryptic peptides account for the composition of the hormone. All tryptic peptides have been completely sequenced except T12 which has 31 amino acids with the following composition: Lys (1) His (1) Asp (6) Ser (1) Glu (4) Pro (2) Gly (5) Ala (6) Val (1) Leu (4).

In order to obtain the sequence of T12, it was necessary to isolate the peptide by submitting the tryptic digest of β -LPH (17 mg) on Sephadex G-25 (fine) in 0.1 M HOAc. The material in the second peak was recovered by lyophilisation and shown to be peptide T12 with a yield of 3.3 mg. Two samples of T12 (1 mg) were digested separately by pepsin and thermolysin; the digests were mapped and eluates of each spot were subjected to amino acid and sequence analyses. For the determinations of Asp/Asn and Glu/Gln, amino acid analysis of the leucineaminopeptidase and electrophoretic mobility of pH 6.7 on paper were used.

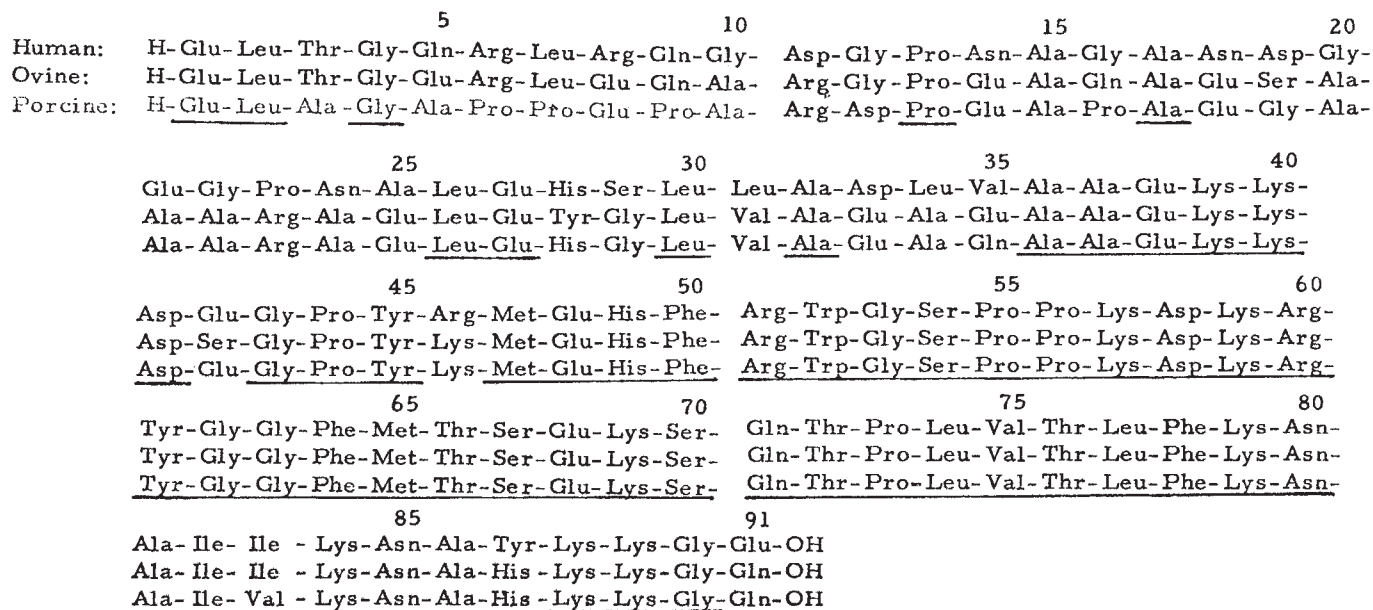
The following chymotryptic peptides (see Fig. 1) were obtained by two dimensional chromatography-electrophoresis of the chymotryptic digest (1 mg) in order to align the tryptic peptides: C17B, C8, C18, C23, C4, C16, C5, C15, and C2. These chymotryptic peptides were analysed for amino acid composition and partially sequenced. From these data, the complete amino acid sequence of β -LPH is proposed as shown in Fig. 1.

The sequence of COOH-terminal 52 amino acid residues

Fig. 1 Amino acid sequence of human β -lipotropin.

has been reported by Cseh *et al.*²⁰. In addition, these investigators indicated that their β_h -LPH preparation has the NH_2 -terminal sequence: NH_2 -Glx-Gly-Asx-. Our data, shown in Fig. 1, are in agreement with the Hungarian workers on the COOH-terminal sequence but differed on the sequence at the NH_2 -terminus.

From a comparison of the proposed structure of β_h -LPH with the lipotropin sequences of other species⁹⁻¹², it is evident that the sequence of COOH-terminal 56 residues is surprisingly homologous whereas the amino acid sequence at the NH_2 -terminus exhibits considerable variability as shown in Fig. 2.

Fig. 2 Comparison of the amino acid sequence of β_h -LPH with that of the ovine and porcine hormone.

We thank Jean Knorr and Kenway Hoey for technical assistance. This work was supported in part by the US National Institutes of Health.

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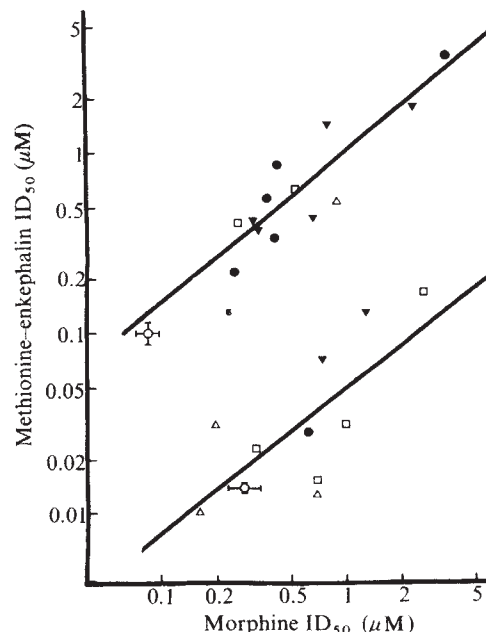


Fig. 1 Correlation between ID_{50} values of morphine and methionine-enkephalin. Myenteric plexus-longitudinal muscle preparation of the guinea pig ileum (upper line) and mouse vas deferens (lower line) from controls and animals implanted with pellets of morphine base. Controls, ○; implanted animals, tissues not preincubated with morphine, □; implanted animals, tissues preincubated with morphine, △ 0.5 μ M, ▽ 1 μ M, ● 2 μ M. The lines were drawn from calculated regressions, the slope of the upper line being $b = 0.84$ and that of the lower line $b = 0.79$.

Cross tolerance between morphine and methionine-enkephalin

TOLERANCE and dependence are characteristic symptoms in man during prolonged use of opiates. We have pointed out¹ that for an understanding of these phenomena the interaction between endogenous enkephalin and exogenous opiates is of importance. Normally, enkephalin can be assumed to control inhibitory mechanisms determining the rate of neurotransmitter release. If, however, opiates are administered with the intent of increasing the effects of these inhibitory mechanisms, then control will pass from the endogenous enkephalin to the exogenous opiates. Since tachyphylaxis is a pharmacological characteristic of opiates, a state of tolerance will arise in which increasing amounts of opiates will be required to maintain the desired effect. As an important step in the elucidation of the mechanism of action of enkephalin, it was necessary to establish whether cross tolerance between enkephalin and opiates is found in morphine-tolerant animals. This point was tested in two morphine-sensitive models, the myenteric plexus-longitudinal muscle preparation from the guinea pig ileum^{2,3} and the mouse vas deferens⁴. As natural enkephalin consists of about 75% methionine-enkephalin and 25% leucine-enkephalin⁵, the relative potencies of synthetic methionine-enkephalin and morphine were compared in preparations obtained from animals with different degrees of tolerance. Cross tolerance between morphine and methionine-enkephalin was shown to occur in the two preparations obtained from animals implanted with morphine pellets.

Mice were implanted with 1 or 2 pellets, each containing 75 mg morphine base, and guinea pigs with 2 or 4 pellets. After 3 d the animals were killed; the myenteric plexus longitudinal muscle² was prepared for isometric recording or the vas deferens⁴ for isotonic recording. The bathing medium contained either no morphine or, to imitate the *in vivo* conditions, 0.5, 1 or 2 μ M morphine. After approximately 2 h of exposure, the morphine was washed out and, when the sensitivity to morphine or methionine-enkephalin

stopped increasing, dose-response curves for morphine and methionine-enkephalin were obtained and the ID_{50} values calculated. Preincubation with morphine had no consistent effect on the ID_{50} values.

The results (Fig. 1) show that in both preparations tolerance of varying degree had developed. In the ileum from control guinea pigs the ID_{50} of morphine was 94.4 ± 13.2 nM and that of methionine-enkephalin 114.7 ± 19.7 nM, with a potency ratio of enkephalin to morphine of 0.89 ± 0.08 ($n=12$). In the preparations from pellet-implanted guinea pigs, the ID_{50} values were increased 3 to 400-fold but the mean potency ratio of 0.97 ± 0.10 ($n=13$) did not differ from that found in the controls. Similar results were obtained for the mouse vas deferens, although the correlation was not as good as in the myenteric plexus and there was an overlap of the ID_{50} values of preparations from pellet-implanted and non-implanted mice. The potency ratio of enkephalin to morphine was 21.5 ± 4.6 ($n=4$) in the controls and 22.9 ± 5.3 ($n=10$) in preparations from pellet-implanted mice.

The molecular mechanisms underlying cross tolerance are uncertain. Since the number of receptor sites in brain or their affinity to opiates are the same in normal and morphine-tolerant rats⁶, tolerance and, therefore, also cross tolerance may be due to a process subsequent to the activation of the recognition sites of the receptor complex. This possibility is supported by the observation that the myenteric plexus-longitudinal muscle preparation obtained from morphine-tolerant guinea pigs shows reduced sensitivity to the depressant effects not only of morphine but also of adrenaline and noradrenaline^{7,8}. The importance of this observation lies in the fact that the depressant effect of catecholamines is due to activation of presynaptic α -adrenoceptors⁹ which are different and separate from opiate receptors¹⁰. It is possible that changes in adenylate cyclase activity^{11,12} represent the common factor in the simultaneous changes in the sensitivity to opiates and catecholamines.

This work was supported by grants (to H.W.K.) from the US National Institute on Drug Abuse, the Medical Research Council and the US Committee on Problems of Drug Dependence, NAS-NRC. We thank Dr B. A. Morgan (Reckitt and Colman) for synthetic methionine-enkephalin.

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Analgesia induced *in vivo* by central administration of enkephalin in rat

ENKEPHALIN, a natural opiate receptor agonist in brain¹⁻⁴, has been identified by Hughes *et al.*⁵ as a mixture of two pentapeptides: H-Tyr-Gly-Gly-Phe-Met-OH (methionine-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (leucine-enkephalin). Both peptides mimic the ability of morphine to block electrically evoked contractions of mouse vas deferens and guinea pig ileum, and both inhibit the stereospecific receptor binding of the opiate antagonist ³H-naloxone in brain homogenates⁵. On the basis of this and other⁶⁻⁸ evidence, it has been proposed^{9,10} that enkephalin receptors may be sites at which morphine-like drugs exert their analgesic actions, and that the enkephalins themselves may be modulators or transmitters in brain systems for pain suppression or analgesia. Consistently with this suggestion, we find that methionine-enkephalin and leucine-enkephalin, when administered through permanently indwelling cannulae in the lateral ventricles of rats, induce a profound analgesia *in vivo* that is fully reversible by naloxone.

Methionine-enkephalin and leucine-enkephalin were synthesised by the solid phase method. After purification by gel filtration, homogeneity of the peptides was verified by amino acid analysis and thin-layer chromatography. Intraventricular cannulae, constructed from 27-gauge stainless steel tubing, were implanted in 300-400-g male Charles River rats under pentobarbital anaesthesia, as described previously¹¹. After a recovery period of at least one week, the rats were tested in the tail-flick procedure of D'Amour

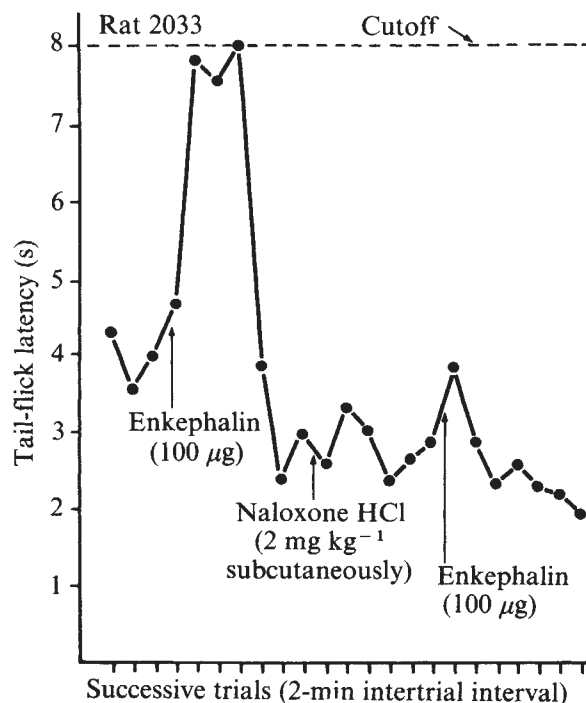


Fig. 1 Analgesic effect of intraventricularly-administered methionine-enkephalin and its prevention by subcutaneous administration of naloxone in a representative rat. Note especially the brief delay in onset of the first enkephalin response, and the apparent absence of effect on response latency of naloxone itself.

and Smith¹², an analgesic test with good selectivity for opiate drugs¹³. Animals were comfortably restrained in a wire-mesh cylindrical chamber so that the tip of the tail could be positioned at the focal point of a radiant heat source (1183 GE lamp). After an acclimatisation period of 15 min, the lamp was turned on and an electric clock was activated. Movement of the tip of the tail exposed a photocell to the lamp, which turned off the thermal stimulus and stopped the clock. If the rat failed to move its tail within 8 s, the heat was automatically turned off to prevent blistering. The intensity of the lamp was adjusted in each case to produce at least three baseline tail-flick latencies of 2.5-5 s. Tests were made every 2 min.

After preliminary experiments to establish the effective dose range, 100 or 200 µg of methionine-enkephalin or leucine-enkephalin was dissolved in 10 µl of Ringer's solution and injected in the lateral ventricle at a rate of 0.2 µl s⁻¹. Injections of Ringer's solution at pH 4.0 (to approximate the pH of the enkephalin solutions) served as control. To minimise disturbance of the rats, connections to the intraventricular cannula were made before the baseline tests. Behavioural reactions to the injection itself were rarely observed.

Statistically significant increases in tail-flick latency

Table 1 Analgesic activity of enkephalin and morphine as assessed by an increase in the latency of a tail-flick response to noxious thermal stimulation in different groups of rats

Drug	Dose (µg)	No. of rats	Mean latency ± s.e.m.		Mean % change ± s.e.m.
			Baseline	Drug	
Ringer's (pH 7.4)		10	3.73 ± 0.27	3.94 ± 0.25	7.4 ± 6.2
Ringer's (pH 4.0)		13	4.02 ± 0.20	4.27 ± 0.28	6.6 ± 6.9
Morphine sulphate	10	7	3.96 ± 0.42	6.59 ± 0.73†	65.4 ± 9.1
Methionine-enkephalin	100	24	3.83 ± 0.14	5.19 ± 0.31†	37.2 ± 8.8
	200	3	2.88 ± 0.41	4.99 ± 0.85*	77.8 ± 35.8
Leucine-enkephalin	100	11	3.97 ± 0.17	5.11 ± 0.34*	31.7 ± 11.5
	200	4	3.35 ± 0.35	5.54 ± 0.72†	70.5 ± 29.3

*Drug minus baseline significantly different from Ringer's (pH 4.0) minus baseline, $P < 0.05$.

† $P < 0.01$.

All drugs were dissolved in 10 µl of Ringer's solution and injected in the lateral ventricle through permanently-indwelling cannulae. Numbers give mean latencies of three "baseline" and five "drug" responses recorded consecutively at 2-min intervals just before and after injections.

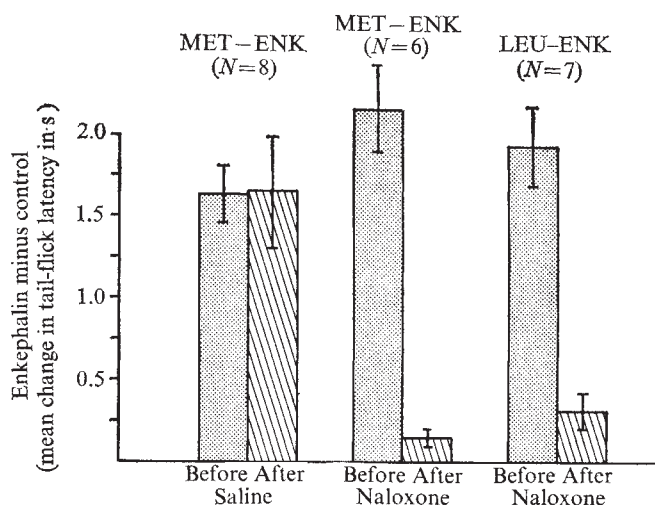


Fig. 2 Summary data of naloxone-reversal experiments. Methionine-enkephalin (MET-ENK) or leucine-enkephalin (LEU-ENK), in intraventricular doses of 100 or 200 μ g, was administered both before (stippled) and 12–16 min after (hatched) subcutaneous injections of 2 mg kg^{-1} of naloxone (or saline). Analgesic responses to both peptides were significantly ($P < 0.025$) reduced following naloxone. Bars indicate standard errors.

followed injections of both peptides (Table 1); in most cases, the 8-s cutoff was reached at the peak of the drug action. The analgesic effect developed rapidly after a definite latent period (2–6 min) and dissipated rapidly (10–12 min) (Fig. 1). Although systematic dose–effect data were not obtained, the larger dose of both substances produced a stronger effect. Methionine-enkephalin and leucine-enkephalin were similar in potency, but each was less potent than morphine by an order of magnitude or more. The analgesia caused by intraventricular administration of 10 μ g of morphine sulphate matched in intensity and reliability that induced by 200 μ g of the enkephalins, although the effects of morphine lasted substantially longer (>1 h). The weaker potency and shorter duration of action of the natural substances may be attributed at least in part to their relatively rapid degradation by brain enzymes². Slow permeation of the peptides to deeply situated receptor sites, as suggested by the delay in onset of action, would further favour their inactivation.

Blockade of the analgesic response by naloxone was evaluated in 13 rats that had exhibited a reliable effect to either methionine-enkephalin or leucine-enkephalin. After the effects of the first dose of enkephalin had clearly worn off, 2 mg kg^{-1} of the opiate antagonist was administered subcutaneously. 12–16 min later, at the approximate peak of the action of naloxone, the enkephalin dosing was repeated. In all naloxone-treated rats, the analgesic response to the second injection of peptide was virtually abolished (Fig. 2). In control experiments, subcutaneous administration of saline in place of naloxone was shown to have no measurable effect on the response to methionine-enkephalin.

Our observations suggest that enkephalin has opiate-like analgesic properties. In pharmacological studies of pain perception, however, especially when a substance is injected directly in the brain, serious consideration must be given to the possibility of artefacts. This possibility seems remote in the present case for several reasons. First, the detailed pattern of analgesic activity after systemic administration of morphine is reproduced by microinjections of the drug into the ventricles or central grey matter of the brain^{7,8,14}. Second, as already indicated, the D'Amour-Smith test is highly selective for opiate-like activity and tends to yield negative results for other drugs, even non-opiate analgesics. Finally, naloxone blockade may be taken as a specific indication that enkephalin induces analgesia by activation of opiate receptors. We therefore conclude that our findings lend support to the concepts of Hughes² and Liebeskind

*et al.*⁷ that enkephalin may serve as a natural opiate-like neurohormone in a central mechanism for the inhibition of pain.

We thank Herman Morris and William Carmint for technical assistance.

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Inability of bats to synthesise L-ascorbic acid

RECENT speculations on the loss and retention of the ability to synthesise L-ascorbic acid (vitamin C) during evolution of animals have been based on few data, often for limited numbers of species¹. Published reports for the Mammalia deal mostly with domesticated and laboratory species, and there is little information on ascorbate biosynthesis in wild populations. The status of knowledge on bats typifies this paucity of data. It has been reported that two species of bats (of approximately 850 species), like anthropoid primates and guinea pigs, are unable to synthesise vitamin C because they lack L-gulonolactone oxidase². When present, this enzyme catalyses the final step in the biosynthesis of L-ascorbic acid from glucose. These species are *Pteropus medius*³ (= *P. giganteus*), a fructivore of the suborder Megachiroptera, and *Vesperugo abramus*^{2,4} (presumably *Pipistrellus coromandra*), an insectivore of the only other chiropteran suborder, the Microchiroptera. We have assayed livers of 34 species of New World microchiropteran bats for L-gulonolactone oxidase (Table 1). These species represent six families, temperate and tropical environments, and a wide variety of food habits and life histories. Within the limits of our assay, we could not detect L-gulonolactone oxidase in livers of any of the species examined. This greatly strengthens the hypothesis that all members of the Chiroptera lack the ability to synthesise vitamin C, which is surprising considering the diversity of bats and their many highly specialised diets.

L-Gulonolactone oxidase has been detected in the microsomal fraction of liver cells⁵ in all mammals shown to synthesise ascorbate. Most previously reported investigations involved a technique in which microsomes are prepared from liver homogenate by differential centrifugation, and only the microsomal fraction is analysed for the enzyme⁵. We have shown, however, that a large portion of the enzyme may remain with other fractions and thus not be recovered with the microsomes⁶. Enzyme activities for *Pteropus* and *Vesperugo* were reported^{3,4} as “nil”.

Although these workers probably used a quantity of microsomal preparation equivalent to 0.25 g of liver in the assay, the sensitivity of their method cannot be assessed adequately because of the potential for loss of any existing L-gulonolactone oxidase during preparation of the microsomes.

A more sensitive method⁶, in which essentially all the

L-gulonolactone oxidase is solubilised by homogenising liver tissue in a medium containing sodium deoxycholate, was used for the assays reported here. This method detects lower levels of L-gulonolactone oxidase in liver than does the microsomal method. When assays are conducted on individuals the technique may be limited by the small size

Table 1 Bats examined for L-gulonolactone oxidase in the liver

Species examined	No. and sex of specimens		Specimen origin*	L-Gulonolactone oxidase activity†
	♂♂	♀♀		
Family Noctilionidae				
<i>Noctilio leporinus</i>	2		Tropics	< (0.06–0.07)
Family Mormoopidae				
<i>Pteronotus davyi</i>	2		Yucatan	< 0.07
<i>Pteronotus parnellii</i>	2	3	Tropics	< 0.09 (0.07–0.11)
<i>Pteronotus suapurensis</i>	1		Costa Rica	0.18
<i>Mormoops megalophylla</i>	1		Yucatan	< 0.08
Family Phyllostomatidae				
Subfamily Phyllostomatinae				
<i>Micronycteris megalotis</i>	1		Yucatan	< 0.09
<i>Mimon cozumelae</i>	1		Yucatan	< 0.09
Subfamily Glossophaginae				
<i>Glossophaga soricina</i>	9		Tropics	< 0.20 (0.07–0.30)
<i>Glossophaga soricina</i>		9	Costa Rica	< 0.18 (0.08–0.30)
Subfamily Carollinae				
<i>Carollia brevicauda</i>	3		Tropics	< 0.11 (0.08–0.14)
Subfamily Sturnirinae				
<i>Sturnira lilium</i>	1		Quintana Roo	< 0.07
<i>Sturnira ludovici</i>	1	1	Costa Rica	< (0.06–0.13)
Subfamily Stenoderminae				
<i>Uroderma bilobatum</i>	1	1	Costa Rica	< (0.08–0.14)
<i>Chiroderma villosum</i>	1		Quintana Roo	< 0.09
<i>Artibeus jamaicensis</i>	4	13	Tropics	< 0.07 (0.03–0.14)
<i>Artibeus lituratus</i>	2	3	Costa Rica	< 0.07 (0.05–0.09)
<i>Artibeus phaeotis</i> (infant)	1		Quintana Roo	< 0.15
<i>Artibeus toltecus</i>	1	3	Costa Rica	< 0.10 (0.06–0.14)
Subfamily Desmodontinae				
<i>Desmodus rotundus</i>		1	Yucatan	< 0.07
<i>Diphylla ecaudata</i>		1	Yucatan	< 0.09
Family Natalidae				
<i>Natalus stramineus</i>	1		Yucatan	< 0.20
Family Vespertilionidae				
<i>Myotis keaysi</i>	1		Yucatan	< 0.20
<i>Myotis leibii</i>		3	South Dakota	Nil‡
<i>Myotis leibii</i> (infants)	3	2	South Dakota	Nil‡
<i>Myotis lucifugus</i>	3	16	Minnesota	Nil‡
<i>Myotis lucifugus</i>	1		Minnesota	< 0.18
<i>Myotis velifer</i>	1		Kansas	< 0.30
<i>Myotis riparius</i>		1	Costa Rica	0.51
<i>Eptesicus fernalis</i>	1		Yucatan	< 0.14
<i>Eptesicus fuscus</i>	8	6 (+ 3 unknown)	Minnesota	< 0.07 (0.05–0.25)
<i>Lasiurus ega</i>		2	Yucatan	< (0.08–0.14)
<i>Lasiurus intermedius</i>		1	Quintana Roo	< 0.18
<i>Plecotus townsendii</i>	1		Montana	< 0.30
Family Molossidae				
<i>Molossus ater</i>		1	Yucatan	< 0.08
<i>Molossus sinaloae</i>	1		Yucatan	< 0.07
<i>Promops centralis</i>	1		Yucatan	< 0.09
<i>Eumops glaucinus</i>	1		Yucatan	< 0.09

* Bats were obtained from natural habitats in Yucatan and Quintana Roo, Mexico in April and May, Costa Rica in August and September and various places within the USA primarily during summer. Locality of collection sites is specified to state or country, except where our values represent specimens from two or more tropical localities, in which case locality is designated tropics. Livers were removed immediately after killing, frozen quickly and shipped and held in the frozen state until assayed. Voucher specimens of all species are preserved in the Bell Museum of Natural History.

† Analysed by method of Ayaz *et al.*⁶ except as noted, and expressed as μmol ascorbate per g liver per h. Individual whole livers weighing 0.06–1.00 g were homogenised in 5–10 ml of buffer containing 0.2% sodium deoxycholate. The homogenate was centrifuged at 20,000g for 30 min and sample of the supernatant was incubated for 30 min with L-gulonolactone (5.6 mM). We encountered no interference attributable to lactonases present in the homogenate⁶, and addition of sodium pyrophosphate⁵ did not affect the apparent activity of the enzyme. Ascorbate produced was estimated by the Roe–Kuether colorimetric method. Blanks were made by omitting L-gulonolactone from the incubation mixture. The net absorbance (sample minus blank) was above the value of 0.01, which we consider the minimum absorbance detectable with precision, for only three specimens (0.011, 0.025 and 0.011 for *Pteronotus suapurensis*, *Myotis riparius* and one *Artibeus jamaicensis*, respectively). Calculated values of L-gulonolactone oxidase activity are presented for these three; for all others the net absorbance was less than 0.01 and activities are reported (individual values or means and ranges) as falling below the values calculated for the respective liver weights if the net absorbance has been 0.01.

‡ Analysed by microsomal technique of Chatterjee⁵.

of some bat livers. Consequently, we cannot deny unequivocally the presence of enzyme at levels up to the values given in Table 1. These values, however, are similar to those calculated in like manner for guinea pigs⁶, a species classically considered to lack the ability to synthesise ascorbate⁷.

We do not think that the apparent lack of enzyme activity shown in Table 1 resulted from loss in storage of the livers before analysis because some livers were analysed immediately after removal and others were transported and stored with rodent livers, which invariably exhibited activity levels normal for the species. No relationship was observed within the Chiroptera for change in biosynthetic ability with age (infant *Myotis leibii* and *Artibeus phaeotis* did not have L-gulonolactone oxidase) or sex (note especially *Glossophaga soricina* in Table 1).

Giroud *et al.*⁸ demonstrated the presence of ascorbate in adrenals, kidney, and liver of bats of the genera *Plecotus*, *Rhinolophus* and *Eptesicus*. We calculated mean ascorbate levels in mg per g tissue for five pooled samples of each tissue from ten young-of-the-year and two adult *Eptesicus fuscus* caught in late August as follows: adrenal, 0.29; spleen, 0.31; kidney, 0.10; liver, 0.20; testes (seven males), 0.19. Blood of the same bats contained 0.6–0.9 mg ascorbate per 100 ml. These values are comparable with those of mammals known to be synthesisers⁸, and together with those for *Myotis lucifugus*⁹ they demonstrate that bats are neither deficient in ascorbate nor adapted to survival in the absence of that molecule.

We investigated the hypotheses (1) that bats synthesise ascorbate in some tissue other than liver or (2) that they do so in liver *in vivo* but the reaction is somehow inhibited *in vitro*. Pooled samples of kidney tissue of *Myotis lucifugus* and *Eptesicus fuscus* were examined for L-gulonolactone oxidase using the microsomal⁵ and deoxycholate⁶ techniques, respectively. No enzyme activity was detected for either species. To test for an inhibitor, an homogenate of liver of *Eptesicus fuscus* was mixed 5:1 with one of rat. The enzyme activity of the rat liver was 12.4 μmol per g liver per h when assayed alone and 12.9 μmol per g liver per h when assayed in the mixture with bat liver. The possibility that bats synthesise ascorbate by a pathway that does not involve L-gulonolactone oxidase was not investigated.

The lack of L-gulonolactone oxidase in bat liver is strong presumptive evidence of lack of ability to synthesise L-ascorbic acid. Evidence of deficiency on feeding ascorbate-free diets would provide confirmation, but all previously confirmed cases of lack of synthetic ability in vertebrates are due to absence of L-gulonolactone oxidase. We conclude therefore that the bats examined do not synthesise ascorbic acid, and we anticipate that this will prove to be characteristic of all members of the Chiroptera.

In spite of some speculation^{10,11}, the selective pressures that have resulted in the loss of L-gulonolactone oxidase on at least three occasions during the evolution of the Mammalia are unknown, and not appropriate for discussion in this brief report. Regardless of the specific cause, the ability to synthesise an essential molecule should be lost only when an adequate supply of that molecule is available in the diet. It might then be predicted that species unable to synthesise would have limited potential to exploit new habitats and food sources and ultimately to radiate phylogenetically. Accordingly, the guinea pig is the only known non-synthesising rodent, and the anthropoid apes are relatively limited in number of species and generally are herbivorous or omnivorous. Such is not the case for bats. Within our sample alone, species are represented whose primary foods are fish (*Noctilio*), fruit (*Artibeus* and others), pollen-nectar (*Glossophaga*), blood (the desmodontines) and insects (the mormoopids, natalids, vespertilionids and molossidids).

The earliest bats were probably primarily insectivorous¹². Although some chiroptologists would disagree and little fossil evidence exists, bats are most likely monophyletic—they have evolved from some common non-synthesising, insectivorous stock. It follows then that lack of the ability to synthesise L-ascorbate has not been an evolutionary handicap for many bat lineages. Bats may have selected as primary diets foods high in ascorbate, or they may be especially adapted to utilise ascorbate efficiently, or both. One obvious food resource known to be deficient in ascorbate is seeds¹³, on which no bat relies for a primary dietary item.

Field work in Yucatan was supported by funds from the Society of the Sigma Xi and College of Biological Sciences of the University of Minnesota. K.M.A. was supported by a training grant from the National Institutes of Health. Robert M. Timm assisted us in the field and Peter Gonnella assisted with the assays.

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Received September 8, 1975; accepted February 23, 1976.

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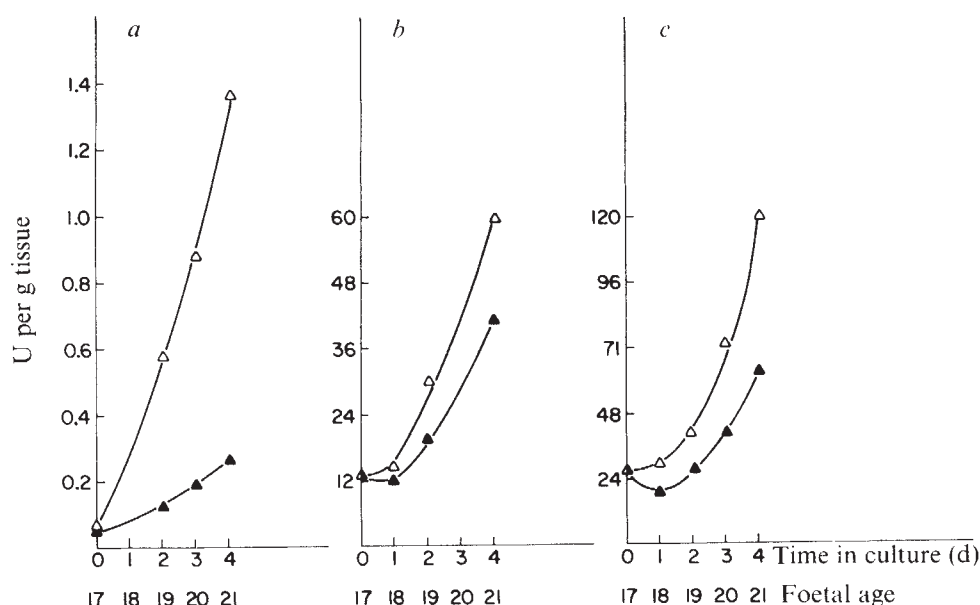
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Interaction of Walker 256 mammary carcinoma and foetal rat liver in organ culture inhibits enzyme maturation

WHEN tumour tissue is implanted subcutaneously into an animal the enzymatic composition of the host liver changes so as to resemble that of well differentiated hepatomas^{1–4}. When a tumour is implanted into a suckling rat (14–20 d old) the appearance of liver enzymes that occurs normally at that stage of development is severely inhibited⁴ by as yet unknown mechanisms. We have investigated whether possible endocrine modification of the host by the implanted tumour is an obligatory step in the enzymatic modification of the host liver. We used cultured foetal liver, a system in which several enzymes have been reported to arise spontaneously^{5–7}. Our results show that tumours can exert a direct effect on enzyme maturation of foetal liver in organ culture that is quantitatively comparable with the effect observed *in vivo*; they do not rule out a possible glandular involvement of the tumour-bearing host *in vivo*.

Livers from rat foetuses at 16–19 d gestation were removed aseptically, cut into 1–2 mm³ fragments, rinsed with Waymouth 751 medium and placed in groups of eight on top of 25 mm Metricel membrane filters, type GA-4 (0.8 μm), supported by sterile Gelfoam sponges (Upjohn) previously saturated with medium. Two such arrangements were maintained per 60-mm Falcon plastic culture dish. The solid transplantable mammary carcinoma Walker 256 was treated in the same way

Fig. 1 Enzyme development in 17-d foetal liver explants. Falcon plastic dishes (60 mm) contained 4 ml Waymouth 751 medium supplemented with 10% heat-inactivated horse serum, 100 U of penicillin, 50 μ g of streptomycin, 5 μ g of fungizone per ml and, where indicated, with hydrocortisone ($0.4 \mu\text{g ml}^{-1}$). The pH of the medium was adjusted to 7.4 by addition of a 7.5% sodium bicarbonate solution and the dishes were incubated in Torbal anaerobic jars at 37°C in an atmosphere of 70% oxygen, 5% CO_2 , 25% nitrogen for a maximum of 5 d with daily changes of the medium. $\blacktriangle$, No additions to culture medium; $\triangle$, hydrocortisone ($0.4 \mu\text{g per ml}$), TAT; b , AAT; c , arginase



except that it was placed directly on to the sponges covered by the Metrical filters that supported the liver explants. Microscopy showed that the liver explants were not invaded by the tumour tissue and that the Metrical membranes prevented migration of cellular outgrowth. The viability of the tumour tissue was assessed by following the specific activity of thymidine kinase (EC 2.7.1.21) assayed by the method of Klemperer and Hayes⁸, and that of the liver explants by following the accumulation of three enzymes: tryptophan α -ketoglutarate transaminase (TAT) (EC 2.6.1.5) assayed by the method of Lin *et al.*⁹, aspartate aminotransferase (AAT, EC 2.6.1.1) assayed according to Herzfeld and Greengard¹⁰ and arginase (EC 3.5.3.1) determined according to Geyer and Dabich¹¹. In addition, the maintenance of total weight (12–20 mg per filter) and total protein (3–5 mg per filter) for the duration of the culture proved to be a sensitive indicator of viability.

The development of AAT and arginase in the presence and absence of hydrocortisone in 17-d foetal liver explants is shown in Fig. 1. The kinetics of accumulation of these enzymes were similar in explants of 16-d-old foetuses but absolute values were about half of those in 17-d-old embryos. When livers of 19-d-old foetuses were explanted, initial values were higher but rose less sharply during culturing. The response of

all three enzymes to hydrocortisone in the medium was the same at all ages. Although animals were time-mated, an 8–10 h uncertainty with respect to age (several litters were needed for each of the experiments reported) was sufficient to make standard deviations seem larger than those inherent in either the enzyme assays or the development of the enzymes in duplicate explants from the same litter. Representative data are therefore shown, and when tumour tissue was cocultured with foetal liver (below) experimental and control explants were from the same litter.

The behaviour of AAT in organ culture is similar to that *in vivo*: measurable levels appear on day 17 of gestation and a significant increase in activity has been reported in response to hydrocortisone¹⁰. The behaviour of TAT and arginase, on the other hand, differs from that *in vivo*: TAT does not appear until after birth, cannot be induced prematurely by hydrocortisone, and appears prematurely only when the young are delivered surgically before term¹². Arginase activity can be detected from day 16 of gestation, but cortisol had no enhancing effect, during the prenatal period¹³.

When foetal livers were cocultured with Walker 256 carcinoma (Fig. 2) the rate of accumulation of TAT decreased by 40%, and that of AAT by 25%. The development of arginase

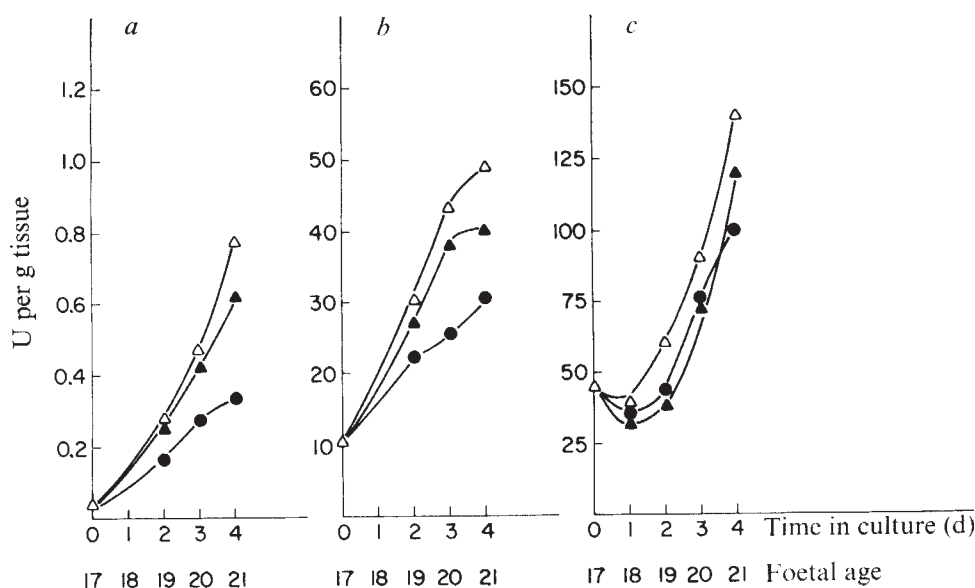


Fig. 2 Enzyme development in 17-d foetal liver explants cultured in the presence of Walker 256 mammary carcinoma. Livers were explanted as described for Fig. 1. All cultures were supplemented with 2 mM glutamine and hydrocortisone ($0.4 \mu\text{g ml}^{-1}$). $\blacktriangle$, Foetal liver; $\bullet$, foetal liver in the presence of tumour tissue; $\triangle$, foetal liver in the presence of mammary tissue. a , TAT; b , AAT; c , arginase.

was not affected, precluding the interpretation that the tumour exhausted an essential component of the medium. This possibility is further made unlikely because liver explants cultured in the presence of tumour tissue consistently increased in total mass and protein by 15–20%, whereas controls did not.

All culture dishes containing Walker carcinoma (and those serving as controls) were supplemented with additional 2 mM glutamine (in view of the high glutaminase content of tumours) and with hydrocortisone ($0.4 \mu\text{g ml}^{-1}$). The inhibitory effect of the tumour on the rate of enzyme accumulation in the liver explants did not depend on the presence of the corticoid in the medium; rather, in initial mixed culture experiments without hydrocortisone, enzyme levels in the cocultured liver explants were too low to be measured accurately.

Table 1 Enzyme development in 19-d foetal liver explants

Days in culture	Foetal age	Additions to culture medium			
		None Injected	Controls	Cortisol (0.4 $\mu\text{g ml}^{-1}$) Injected	Controls
TAT					
0	19	0.03	0.03	0.03	0.03
1	20	—	—	—	—
2	21	0.31	0.14	0.74	0.28
3	22	0.54	0.23	1.06	0.67
4	23	0.44	0.39	1.25	0.95
AAT					
0	19	23.7	18.3	23.7	18.3
1	20	—	—	—	—
2	21	48.2	30.0	64.3	42.0
3	22	66.5	41.8	78.1	62.7
4	23	60.5	56.6	92.6	76.0
Arginase					
0	19	26	21	26	21
1	20	37	13	62	47
2	21	90	32	142	90
3	22	83	59	217	158
4	23	70	71	248	174

Foetuses in one uterine horn were injected with $25 \mu\text{g}$ cortisol each, 24 h before their livers were explanted as described in the legend to Fig. 1; non-injected foetuses from the other uterine horn served as controls. Activities are given in units per g tissue.

To ascertain whether the inhibition of TAT and AAT accumulation was tumour specific, foetal livers were cultured in conditions identical to those described, but with mammary tissue from a pregnant dam instead of tumour. The activities of all three enzymes were always 10–20% higher than in controls (foetal liver explants alone). We cannot explain this observation; however, a differentiating effect of mammary mesenchyme on tumour tissue explants has been reported¹⁴.

The inhibitory effect of the tumour on the enzymatic maturation of the liver explants was reversible and depended on the continued survival of the tumour in culture. When the sponges supporting the tumour tissue were replaced by empty sponges on day 3 of culture, enzyme accumulation resumed at the rate of controls. Similarly in some experiments in which the tumour did not survive as long as the liver explants (as ascertained by thymidine kinase activity measurements), accumulation of the indicator enzymes resumed at the rate of controls.

Because the cultures containing the tumour had to be maintained in the presence of hydrocortisone to obtain detectable enzyme levels in the cocultured livers, and all three enzymes responded to the hormone with an increased rate of accumulation in organ culture although two of them did not respond in this way *in utero*, it seemed that the corticoid may serve some nonspecific function in the system, perhaps the maintenance of tissue viability, similar to that of certain serum factors¹⁵. To test this possibility we injected each of several foetuses *in utero* with $25 \mu\text{g}$ hydrocortisone 24 h before their livers were explanted into media with and without hydrocortisone. The results of a representative experiment (Table 1) show that enzyme levels in cultures from 19-d-old foetuses which had received hydrocortisone *in utero* were similar for 2–3 d in culture

to those which were explanted from uninjected controls into medium containing hydrocortisone. The rate of enzyme accumulation in the injected samples then slowed, whereas explants maintained on hydrocortisone with daily medium changes continued to respond with the same rate of enzyme accumulation; those injected and maintained on hydrocortisone showed the greatest response, particularly arginase. When foetuses were injected with hydrocortisone on day 16 of gestation and their livers were explanted 24 h later into media with and without hydrocortisone, the injected hormone was ineffective (data not shown). This argues against the possibility that the injected hormone had some lasting, nonspecific effect; rather it suggests that hydrocortisone has a specific inducing effect if administered when the liver is competent to initiate *in utero* the processes which culminate in enzyme production *in vitro*.

Our findings suggest that the Walker 256 carcinoma produces a diffusable factor (or factors) with an inhibitory effect on the rate of accumulation of some, but not all, enzymes that develop in embryonic liver maintained in organ culture. The effect is specific for Walker carcinoma tissue and its maintenance depends on the continued survival of the tumour in culture. The apparent rapid inactivation of the factor(s) in this system need not signify an inherent instability precluding its isolation; serum factors that withstand remarkably harsh conditions during their purification are known to be inactivated rapidly by cells in culture¹⁶. While the endocrine state of a tumour-bearing animal is undoubtedly modified, our results show that Walker carcinoma tissue exerts a readily measurable direct effect on developing liver in organ culture, that is without the intervention of endocrine changes. The system described is therefore well suited as an assay for the isolation of the pertinent factor(s) and for the study of its mechanism of action.

This work was supported by a grant from the US Public Health Services.

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Mechanism for suppression of cellular biosynthesis of prostaglandins

BIOSYNTHESIS of prostaglandins (PGs) from unsaturated fatty acid precursors involves a complex sequence of reactions that seem to proceed rapidly in response to physiological stimuli. Studies *in vitro* have revealed a capacity for biosynthesis greater than would be expected from measures of tissue content^{1,2} or daily prostaglandin (PG) production³. Biosynthesis requires

release of esterified precursor from tissue lipids^{4,5}, and control of the hydrolytic event may be a major means of controlling PG biosynthesis (for example, in brain⁶ or spleen⁷). Another possible type of regulation is control of cyclo-oxygenase activity. Many chemical agents have been examined as modifiers of the PG-forming oxygenase (for example, reviews in refs 8 and 9). The enzyme can be inhibited by fatty acids¹⁰ although they appear only in limited amounts in the cytosol. In addition, inhibition of the oxygenation reaction has been observed *in vitro* with added glutathione peroxidase (GSP) and reduced glutathione (GSH)¹¹⁻¹⁴. We have further investigated this form of enzymatic regulation, and propose that it inhibits by destroying an essential activator of the oxygenase which forms the PGs and thromboxanes.

Supernatant (BSN) obtained from centrifugation of homogenates of bovine vesicular glands at 105,000*g* for 90 min gave 60% inhibition of oxygenation of arachidonic acid by bovine vesicular gland microsomes, and 40% inhibition of the activity of an acetone powder preparation from sheep vesicular glands. Since the bovine oxygenase showed only 12% of the specific activity of that from sheep, and the supernatant from sheep vesicular glands gave little inhibition of oxygenase preparations, the most active inhibitory and oxygenating preparations were used in subsequent experiments (Table 1). When sodium cyanide, an inhibitor of PG synthesis^{15,16}, was in the oxygenase assay mixture at a concentration (0.5 mM) that gave about 45% inhibition of oxygenation in the absence of added BSN, a synergistic inhibition was observed that was proportional to the amount of BSN when < 0.1 ml of BSN was present, and was complete with ≥ 0.1 ml of BSN. The BSN was only about half as inhibitory when additional GSH was omitted from the assay, and did not inhibit the oxygenase when 1.0 mM *N*-ethylmaleimide (NEM), which combined with the necessary mercaptan group of GSH) was added. Oxygenase activity could also be recovered when NEM was added to a reaction mixture several minutes after total inhibition by 0.1 ml of BSN. The total extent of reaction before and after addition of NEM was the same for all concentrations of BSN used, indicating that the capacity of the oxygenase for production of PGs and related derivatives was not irreversibly impaired by BSN.

When radioactive eicosatetraenoic acid (150 nmol) was incubated with the oxygenase preparation, 69–74% of the product formed migrated with ³H-PGF_{2α} (*R_f* = 0.05–0.20) and ³H-PGE₂ (*R_f* = 0.20–0.45) standards, 12–17% with the region associated with PGD₂, PGG₂ and PGH₂ (*R_f* = 0.30–0.45) and 10–19% with the hydroxy acid standard (*R_f* = 0.45–0.60). The relative amounts of product formed were similar in the presence of GSH and the BSN with or without NEM present. In the presence of 4 mM S_NCl₂, 73–80% of the product

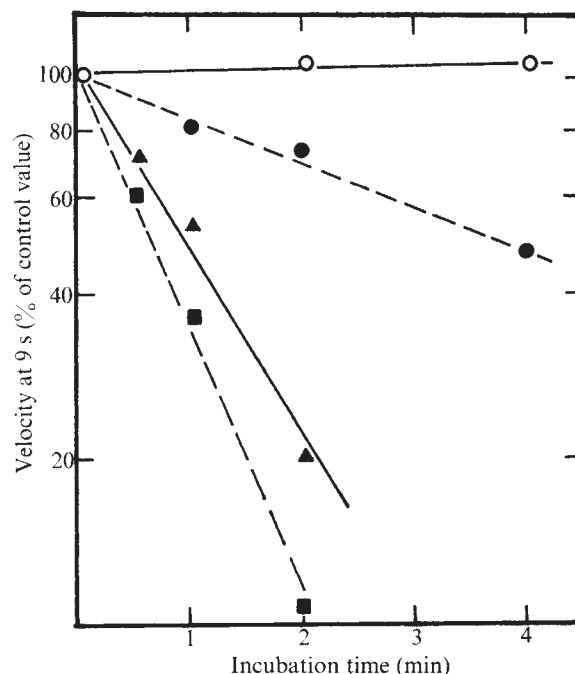


Fig. 1 Time-dependent destruction of the oxygenase activator by BSN and GSP. Activator was generated from 65 μ M arachidonic acid with 350 μ g of sheep vesicular gland dioxigenase in 3 ml of 0.1 M Tris chloride at pH 8.5, containing 0.67 mM phenol and NaCN, as indicated below. After 4 min of reaction and cessation of oxygen consumption, 500 μ M GSH and the BSN or GSP solution were added. Then, after various times, the oxygenase reaction was initiated by addition of 350 μ g of vesicular gland enzyme, and initial velocities were measured using an oxygen electrode equipped with an electronic differentiator. The value for the oxygenase velocity at 9 s is a measure of the amount of activator^{16,18}. $\circ$, 0.5 mM NaCN + heat-treated GSP; $\bullet$, 0.5 mM NaCN + 120 U of GSP; $\blacksquare$, 1.0 mM NaCN + 150 U of GSP; $\blacktriangle$, 0.5 mM NaCN + 0.015 ml of BSN.

migrated with PGF_{2α} (*R_f* = 0.05–0.20) and 6–19% with PGE₂ (*R_f* = 0.20–0.29) standards.

We have proposed^{14,16} that the oxygenase required a hydroperoxide activator and PG production may be inhibited by removal of activator molecules by GSP. Figure 1 shows that very low levels of either BSN or GSP destroy the activator in a few minutes.

The inhibitory agent in the cytosol of bovine glands (BSN) had several properties similar to those of the GSP purified from rat liver¹⁷ or bovine blood¹⁸. Both GSP and BSN were dependent on added GSH for maximal inhibitory ability (and added NEM,

Table 1 Inhibition of sheep vesicular gland dioxigenase by BSN

Initial reaction conditions	After addition of dioxigenase		After addition of 1 mM NEM	
	Optimal velocity (μ mol O ₂ min ⁻¹ mg ⁻¹)	Extent (μ mol O ₂)	Optimal velocity (μ mol O ₂ min ⁻¹ mg ⁻¹)	Extent (μ mol O ₂)
Control	470	46	0	0
+ 50 μ l BSN	430	41	50	7
+ 100 μ l BSN	270	28	145	16
+ 0.5 mM NaCN	289	45	0	0
+ 0.5 mM NaCN + 50 μ l BSN	182	29	74	16
+ 0.5 mM NaCN + 70 μ l BSN	89	19	161	22
+ 0.5 mM NaCN + 100 μ l BSN	0	0	239	38
+ 0.5 mM NaCN + 100 μ l heat-treated BSN	222	32	124	12
+ 0.5 mM NaCN + 100 μ l BSN + 1 mM NEM	318	47	0	0
+ 0.5 mM NaCN + 100 μ l BSN + no exogenous GSH	205	43	0	0
+ 0.5 mM NaCN at pH 7.0	420	42	0	0
+ 0.5 mM NaCN + 70 μ l BSN at pH = 7.0	277	29	17	22

BSN was obtained by homogenising 100 g of bovine seminal vesicles in 200 ml of 0.1 M Tris-HCl at pH 8.5. The homogenate was centrifuged at 10,000*g* for 15 min and the supernatant was filtered through cheesecloth. This supernatant was centrifuged for 90 min at 105,000*g* and the resultant supernatant was removed and stored at -20°C for later assay. Assays were made in 3.0 ml of 0.1 M Tris-HCl at pH 8.5 (except where otherwise indicated the pH 7.0 buffer was 0.1 M EDTA) containing 0.67 mM phenol, 0.5 mM reduced glutathione, 65 μ M arachidonic acid and other additions as indicated. Reaction was initiated by addition of 375 μ g of phenol-activated sheep vesicular gland enzyme. After 3–5 min, 1.0 mM *N*-ethylmaleimide was added to the reaction mixtures. Dioxigenase activity was monitored with an oxygen electrode equipped with an electronic differentiator.

which removes GSH, eliminated inhibition by both); inhibitory in a concentration-dependent manner; synergistic with NaCN in the inhibition of PG formation; heat-labile and non-dialysable, destroying the activator of the oxygenase in a time-dependent manner (Fig. 1).

BSN also appeared most effective as an inhibitor at or near the pH optimum for GSP (pH > 8.5; see also refs 19–22). There was a slight difference in the amount of $(\text{NH}_4)_2\text{SO}_4$ required to salt the two inhibitors out of solution: approximately 68% of the total inhibitory ability of the BSN precipitated from solution between 0.33 and 0.50 saturation with $(\text{NH}_4)_2\text{SO}_4$ whereas about 60% of the measured GSP activity of rat liver supernatant was salted out between 0.45 and 0.65 $(\text{NH}_4)_2\text{SO}_4$. (Rat liver-GSP was obtained by extraction of protein precipitated in ethanol at -25°C (ref. 17), whereas the BSN was a high speed supernatant and contained more protein per ml than the liver preparation which could explain the differences.) BSN, like GSP, catalysed the destruction of cumene hydroperoxide in the presence of reduced GSH (8 U peroxidase activity per μl of BSN—see ref. 18 for assay conditions and definition of units).

The apparent K_m for GSH may vary greatly depending on the initial steady-state concentration of the peroxide substrate^{22,23}, suggesting that as the local physiological concentrations of hydroperoxide substrate approach $1\mu\text{M}$ the *in vivo* level of GSH (usually between 100 μM and 5 mM (refs 24 and 25) may be close to its K_m for GSP. Thus, metabolic alterations of the intracellular concentrations of GSH within this concentration range could alter directly the effective peroxidase activity and thereby act as an intracellular modulator of the activity of the fatty acid oxygenase(s) that form the PGs and thromboxanes. Homogenisation of a tissue *in vitro* also gives dilution of the cytosolic GSP and GSH to concentrations that are less inhibitory than those *in vivo*.

Formation of PGs *in vitro* has been reported to be inhibited in a dose-dependent manner by a component of the supernatant obtained during the preparation of microsomes from bovine seminal vesicles^{26,27} and rabbit renal medulla²⁸, but the nature of the inhibition was unclear. Inhibition by a soluble component from tissue preparations may also be inferred from the data from other studies with these and other tissues^{29–32}, that is, total product found in one or more of the PG fractions was significantly less when supernatant was included in the assays. Metabolic enzymes could lower accumulated levels of PG, particularly where the assay involves measurement of final concentrations of PG products (for example, ref. 32).

Although there is insufficient evidence to propose that GSP is the only inhibitor present in supernatant preparations, many assays for PG biosynthesis have included levels of GSH > 0.2 mM (refs 28–31), and a GSP-like activity could account for much of the inhibitor in supernatant reported in some of those *in vitro* studies. The observed effectiveness of the GSH-dependent peroxidase in blocking PG biosynthesis suggests that it has a significant physiological role in the many cell types in which it occurs. Thus, peroxidase activities could suppress PG biosynthesis by destruction of the essential activator as it is formed, so that (depending on the levels of peroxidase and essential cofactor, GSH) some dioxygenase molecules are not activated and therefore cannot participate in PG production. A decreasing concentration of cellular GSH could be an important means for cells to modulate cyclo-oxygenase activity to give increased production of PGs and their related derivatives.

This research was supported in part by a fellowship (to H.W.C.) from the Medical Research Council of Canada and a grant from the US National Science Foundation.

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Evidence for the proximity of sweet and bitter receptor sites

ALTHOUGH sweetness and bitterness are generally referred to as two of the common or basic tastes, it is by no means certain that they are, in fact, primary¹. Furthermore, neurophysiological evidence has shown that response to several of the basic taste stimuli can occur in a single taste cell². Much structural progress in the chemistry of sweetness has been made in the past decade through Shallenberger's hypothesis^{3–5} that the sweetness of sugars depends on hydrogen bonds between the sugar molecule and receptor site. Our previous work^{6–9} has indicated that sugar molecules and their simple derivatives are nearly always sweet, bitter or bitter-sweet. Bitter-sweet sugar molecules, like certain artificial sweeteners such as saccharin, have both bitterness and sweetness as intrinsic features of their molecules¹⁰—neither response can be altered by exhaustive stepwise purification of such compounds¹⁰. Bitter-sweet sugar molecules appear to be 'polarised' on taste receptors⁹ so that one end of the molecule binds to sweet receptor sites and the other to bitter receptor sites. It is not clear, however, whether these molecules distribute themselves, some on sweet receptors

Fig. 1 Polarisation of methyl- α -D-mannopyranoside molecule on taste receptor.

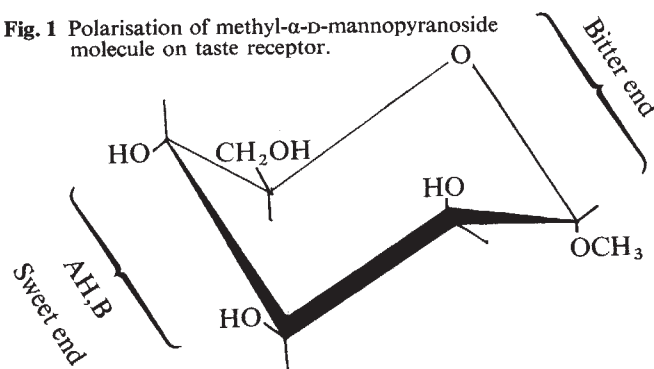


Table 1 Perception of sweetness in sucrose (S) and methyl- α -D-mannopyranoside (M) before and after presaturation of taste receptors with 10 ml 0.0048% quinine sulphate solution*.

	Panellist	IT_{Sa} (7.5%)	IT_{Ma} (10%)	IT_{Sb} (10%)	IT_{Mb} (15%)	IT_{Sc} (12.5%)	IT_{Mc} (20%)	
Before presaturation	A	82	54	—	—	141	164	
	B	51	69	191	189	86	84	
	C	74	69	109	110	117	74	
	D	60	43	140	63	90	250	
	E	39	86	141	117	140	130	
	Mean	61	64	145	120	115	140	
	s.d.	17	16	34	52	26	71	
After presaturation	A	53	13	80	32	140	24	
	B	108	44	83	71	109	85	
	C	190	74	104	87	195	76	
	D	163	76	165	60	173	64	
	E	132	55	55	33	90	34	
	Mean	129	52	97	57	141	57	
	s.d.	41	15	19	24	43	27	
								$IT_{Sa} \vee IT_{Ma}$ $IT_{Sb} \vee IT_{Mb}$ $IT_{Sc} \vee IT_{Mc}$
								NS
								$IT_{Sa} \vee IT_{Ma} P < 0.002$ $IT_{Sb} \vee IT_{Mb} 0.01 < P < 0.05$ $IT_{Sc} \vee IT_{Mc} 0.002 < P < 0.01$

*Sweetness is listed as product of peak subjective intensity (I , arbitrary scale 00 = zero sweetness to 5 = very sweet) and persistence (T) of response for sucrose (S) and methyl- α -D-mannopyranoside (M). Untrained panellists were simply selected on basis of recognising different concentrations employed in this experiment at one sitting. Tap water was used for mouth rinsing and immediately afterwards panellists drank 1.0 ml of either sucrose or corresponding methyl- α -D-mannopyranoside solution, recording their subjective intensities on a Telsac Type X moving chart recorder travelling at 12.0 min⁻¹ as soon as the solution had been imbibed. Only sweetness was recorded, bitterness being ignored. For tasting after presaturation of bitter receptor sites, panellists kept the 10 ml 0.0048% quinine sulphate solution in mouth for 10 s, then spat out, and immediately drank 1.0 ml of either sucrose or corresponding methyl- α -D-mannopyranoside solution, recording subjective intensity and time as before. All recordings were continued until zero sweetness response was obtained. The order of presentation of samples to panellists was randomised. An interval of 1.5 min was allowed between successive corresponding sample tasting, for the comparisons before presaturation, and 5.0 min for comparisons after presaturation. Significance was determined by Student's t test.

and some on bitter receptors, or whether a single molecule can span both sweet and bitter receptor sites simultaneously. We now report an experiment designed to distinguish between these possibilities, using a typical bitter-sweet and conformationally defined glycoside, methyl- α -D-mannopyranoside (Fig. 1.).

The subjective intensity of taste response in sweet¹¹ and bitter¹² molecules is known to be proportional to the logarithm of concentration at presaturation levels^{11,12}. We have examined the persistence time (T) of the response as well as the peak subjective intensity (I) as functions of the concentration of stimulus presented to panellists. The product (IT) of these parameters obtained by a continuous trace on a moving chart recorder is a function of the area under the resulting 'taste' curve, and thus total gustatory response. In Table 1, the sweetness values of sucrose and methyl- α -D-mannopyranoside (measured as the product (IT) at three different concentrations, are listed before and after presaturation of the taste buds with quinine sulphate solution. At all three concentrations tested a significantly diminished

sweetness response after presaturation with quinine sulphate solution is observed with methyl- α -D-mannopyranoside solution, but not with sucrose.

In Table 2 the bitterness values of quinine sulphate and methyl- α -D-mannopyranoside (again measured as the product IT) at two different concentrations are listed before and after presaturation of the taste buds with sucrose solution. At both concentrations a significantly diminished bitterness after presaturation with sucrose solution is observed with methyl- α -D-mannopyranoside but not with quinine sulphate. The results in both tables clearly indicate a lowered gustatory response to methyl- α -D-mannopyranoside by our panellists if their tongues are presaturated with either sweet or bitter substances. Indeed the effect at the lower concentration of bitterness investigated was so marked that four out of the five panellists could detect no bitterness at all after presaturation of their tongues with sucrose, whereas in the same conditions their response to a similarly bitter quinine sulphate solution was little affected.

It is possibly still an open question whether a taste re-

Table 2 Perception of bitterness in quinine sulphate (Q) and methyl- α -D-mannopyranoside (M) before and after presaturation of taste receptors with 10 ml 50% sucrose solution*

	Panellist	IT_Q (0.0016%)	IT_M (3.5%)	IT_Q (0.0024%)	IT_M (4.5%)
Before presaturation	L	190	180	—	—
	M	48	104	48	260
	N	150	145	149	163
	O	23	178	72	186
	P	151	106	90	184
	Mean	112	142	90	198
	s.d.	73	37	43	43
				NS	0.01 < P < 0.05
After presaturation	L	111	0	—	—
	M	58	14	57	56
	N	147	0	148	147
	O	0	0	138	9
	P	42	0	44	209
	Mean	72	3	97	105
	s.d.	58	6	54	90
				P < 0.002	NS

*Bitterness is listed as product of peak subjective intensity (I , arbitrary scale 0 = zero bitterness to 5 = very bitter) and persistence (T) of response for quinine sulphate (Q) and methyl- α -D-mannopyranoside (M). The method used was exactly as before, but for tasting after presaturation, panellists kept 10 ml 50% sucrose solution in mouth for 10 s before spitting out. An interval of at least 20 min was allowed between successive corresponding sample tasting, and significance was again determined by Student's t test.

sponse can be simply and mechanistically explained in terms of bonding forces between a stimulus molecule and a receptor, or whether a more complicated psychological explanation based on the decodifying of perceptual information¹ might give a truer description of the phenomenon. Many advances have been made^{6-9,13-15} on the basis of the former hypothesis, working on the assumption that the sweetness of sugars arises from the existence of AH,B systems in their molecules which govern their ability to hydrogen bond to taste receptors. Sugars generally possess several possible α -glycol groupings meeting Shallenberger's³⁻⁵ requirements for the AH,B system and with oxygen-oxygen interorbital distances of 2.86 Å, but evidence has accrued^{6-9,13-15} to implicate the third and fourth hydroxyl groups of aldohexopyranosyl structures as having a significant and particular role in the sweetness response. By contrast that area of the molecule which surrounds the anomeric centre does not appear to influence sweetness^{6,9,13} but does affect bitterness and these distinct gustatory features seem to be characteristic of a considerable number of different glycoside molecules^{9,13}.

Not all receptor sites are on the same receptor cell, and Ogawa *et al.*¹⁶ have reported that 25% of fibres tested from the chorda tympani of rat and hamster responded to all four basic tests. Frank and Pfaffmann¹⁷ have also shown in the glossopharyngeal nerves of rat that the majority of the fibres are not completely specific for any of the basic tastes, and only a small percentage responded to both sucrose and quinine. It is therefore clear that taste cells may vary in degree of specificity.

The results of this experiment can be interpreted on the basis of our hypothesis that sugar molecules are 'polarised' on taste receptors¹, to mean that the bitter/sweet glycoside attaches itself by a chelating action to the sweet and bitter receptor sites simultaneously. Impairment of either mode of attachment by presaturating the taste receptors with sweet or bitter molecules results in a lowered response. This also suggests that at least some of the sweet and bitter receptor sites might be extremely close to one another, probably within 3-4 Å.

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Received December 15, 1975; accepted February 26, 1976.

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as a function of LDL concentration have led to the conclusion^{1,2} that normal fibroblasts have genetically controlled surface receptors with high affinity for apoLDL and that uptake of LDL by the receptors is essential for the regulation of cholesterol synthesis. These findings raise the possibility that receptor-mediated uptake by fibroblasts, and perhaps by other cells^{1,4}, has an important role in the regulation of apoLDL catabolism and of cholesterol synthesis *in vivo*. But the concentration of LDL protein required to saturate the LDL receptors on cultured fibroblasts (about 50 $\mu\text{g ml}^{-1}$) is less than half the probable concentration in interstitial fluid⁵. Moreover, whereas cultured fibroblasts from patients with familial hypercholesterolaemia (FH) in the homozygous form synthesise cholesterol at 50 times the normal rate, the rate of synthesis of cholesterol in the whole bodies of FH patients is usually normal⁶ or subnormal⁷. For these reasons it seemed desirable to study the binding and catabolism of LDL by uncultured human cells. We used circulating lymphocytes because they can be obtained readily and repeatedly from healthy human subjects. Our results show that uncultured lymphocytes exhibit high capacity surface binding of LDL, but the binding curves show no evidence for the presence of high affinity receptors for LDL.

Lymphocytes, prepared from 300 ml of freshly drawn heparinised blood by density-gradient centrifugation⁸, were washed four times in Eagle's MEM. LDL (density 1.020-1.050), prepared from normal human subjects⁹, was labelled with ¹²⁵I by the iodine monochloride method¹⁰. Of the total ¹²⁵I in the labelled LDL, 5-7% was extractable with chloroform-methanol (2 : 1, v/v).

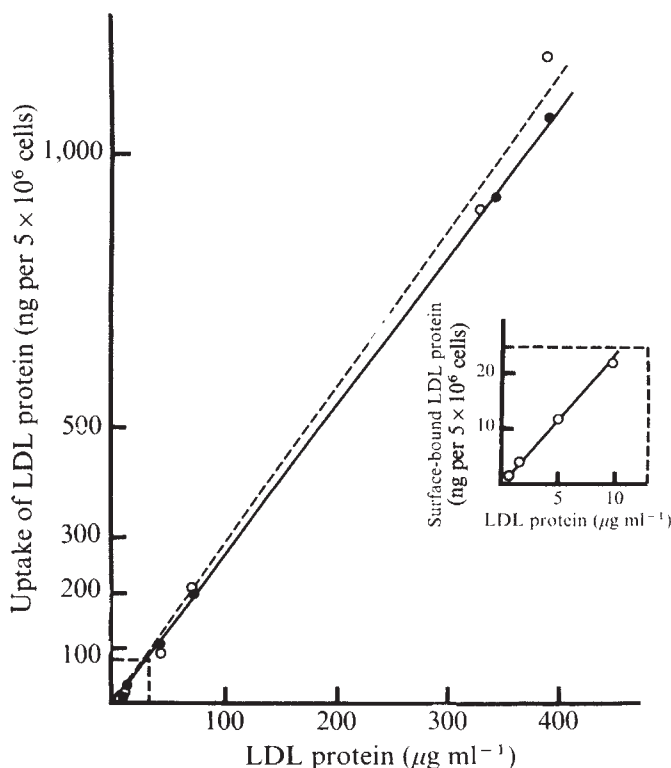


Fig. 1 Uptake of surface-bound (○) and internalised (●) LDL protein by human lymphocytes as a function of LDL concentration in the incubation medium. Inset shows expansion of curve for surface-bound LDL protein at low LDL concentrations. After incubation in duplicate with labelled LDL the cells were washed seven times before radioassay. Surface-bound and internalised LDL protein in the washed cells were estimated from the amounts of non-lipid ¹²⁵I releasable and non-releasable by trypsin respectively. The specific radioactivity of the LDL protein in the incubation medium was used to convert c.p.m. into ng of LDL protein taken up. Values shown are from one of five experiments with lymphocytes from five normal subjects. Experimental details are given in the text.

Uptake and catabolism of low density lipoprotein by human lymphocytes

BROWN and Goldstein^{1,2} have shown that when cultured human fibroblasts are incubated in the presence of low density lipoprotein (LDL), they take up some LDL, the apoprotein of LDL (apoLDL) is catabolised and cholesterol synthesis is inhibited. Experiments on the uptake of LDL

Binding of the labelled LDL by lymphocytes was investigated by incubating suspensions of cells 5×10^6 cells in 1 ml of MEM containing 1% bovine serum albumin (BSA) for 2 h at 25 °C in plastic tubes in the presence of ^{125}I -LDL. The cells were then separated from the medium by centrifugation. Radioactive breakdown products of the labelled apoLDL in the medium were estimated as trichloroacetic acid (TCA)-soluble radioactivity in the supernatant, after correction for radioiodide¹¹. The cells were washed seven times with MEM to remove loosely adsorbed radioactive particles, transferred to fresh plastic tubes and assayed for radioactivity. Lipid-free radioactivity in the washed cells was fractionated into surface bound (^{125}I released by brief incubation with trypsin) and internalised (^{125}I not released by trypsin), essentially by the method of Bierman *et al.*¹¹.

Table 1 Effect of heparin on trypsin-releasable LDL protein bound to lymphocytes

Treatment	Incubation time (h)			
	1	3	4	5
No heparin	244	238	201	213
Heparin at 3.5 h			260	

All values are expressed in ng of LDL protein bound per 5×10^6 cells and are means of duplicate incubations. Lymphocytes were prelabelled by incubation with ^{125}I -LDL at 25 °C for 2 h in MEM containing 1% BSA. Loosely bound LDL was removed from the cells by repeated washing. Duplicate tubes containing the washed labelled cells were incubated for 1, 3, 4 and 5 h. Heparin (10 mg ml^{-1}) was added to one pair of tubes after 3.5 h and the tubes were incubated for a further 30 min. The amount of trypsin-releasable LDL protein bound to the cells at each interval was determined as described in the text. Binding of LDL by cultured fibroblasts has usually been studied at 4 or 37 °C. Our incubations were carried out at 25 °C because at this temperature lymphocytes catabolise apoLDL, while remaining viable for longer than at 37 °C.

When normal lymphocytes were incubated for 2 h with ^{125}I -LDL, uptake of trypsin-releasable LDL protein and of LDL protein not released by trypsin were both directly proportional to the concentration of LDL in the incubation medium over the range from 0.5 to 380 μg of LDL protein per ml (Fig. 1). No inflection in the curve relating uptake to LDL concentration could be discerned, even at the lowest concentrations (Fig. 1, inset). Thus the binding curves obtained in the conditions of these experiments gave no indication of the presence of high affinity LDL receptors with saturation at about 50 μg of LDL protein per ml. In all experiments, about half the total LDL protein taken up by the cells was internalised by the end of the incubation. There was some variation in the amount of LDL protein taken up at a given LDL concentration by lymphocytes from different normal subjects, but almost identical results were obtained when LDL from different subjects was tested against a single preparation of lymphocytes.

The accumulation of TCA-soluble radioactivity derived from the labelled LDL in the medium was determined after 2-h incubations with lymphocytes from normal subjects. In five preparations of lymphocytes the accumulation of radioactive breakdown products, expressed as a percentage of the total cell-associated radioactive protein (surface-bound plus internalised) recovered at the end of the incubation, averaged 4.8 ± 2.0 (s.d.) (range, 1.8–6.9).

The apparent lack of high affinity LDL receptors on lymphocytes could be due to irreversible saturation of binding sites by LDL before the cells are separated from the blood plasma. The binding curves for trypsin-releasable apoLDL, however, suggest that lymphocytes differ from cultured skin fibroblasts in being able to bind LDL to their surfaces by a mechanism that is not saturated at concentrations approaching 400 μg of apoLDL per ml. High capacity binding of LDL by lymphocytes was investigated further in the experiment shown in Table 1. Lymphocytes were incubated for 2 h at 25 °C in MEM containing 1%

BSA and radioactive LDL (500 μg of apoLDL per ml). The cells were then washed seven times and incubated again for increasing periods in MEM containing 1% BSA but no radioactive LDL. Surface-bound LDL protein was assayed at each interval by the methods described above. There was no significant decrease in surface-bound LDL protein during the 5 h of incubation. When heparin (known to displace LDL from the LDL receptors on cultured fibroblasts¹²) was added to the cell suspension after incubation for 3.5 h, there was no displacement of surface-bound LDL protein during incubation for a further 30 min.

These results show that lymphocytes resemble cultured skin fibroblasts in their ability to take up LDL from an incubation medium and to degrade apoLDL. But lymphocytes seem to differ from cultured fibroblasts in being able to bind LDL to high capacity sites that are accessible to trypsin, but from which LDL cannot be displaced by heparin or by incubation in an LDL-free medium for 5 h. A surface-binding mechanism for LDL with high capacity seems more appropriate for circulating lymphocytes than one which is saturated at a concentration of 50 μg of apoLDL per ml, since the concentration of apoLDL in normal human plasma is at least 600 μg per ml.

We thank Mr P. Mistry and Mr D. N. Rudra for assistance.

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Received January 8; accepted February 18, 1976.

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Nuclear retention of receptor-oestrogen complex and nuclear acceptor sites

OESTROGEN-SENSITIVE cells contain cytoplasmic macromolecules called receptors^{1,2}. They bind oestrogen to form complexes which are translocated to the nucleus where they may bind to specific acceptor sites^{3–5}. This interaction of receptor-hormone complex with nuclear acceptor sites is assumed to be responsible for the stimulation of RNA, DNA and protein synthesis that results in growth of the tissue^{3–5}. The demonstration of nuclear acceptor sites has been difficult and controversial^{6,7}. We present here evidence that a limited number of receptor-oestrogen complexes are bound in the nucleus of rat uterine cells and that these sites may be specific nuclear acceptor sites.

Several investigators have shown that nuclear binding of receptor steroid complexes is a saturable phenomenon, thus suggesting that specific acceptor sites exist^{7–14}, while others claim that limited numbers of specific nuclear sites do not exist and that nuclear saturation is an artefact^{6,15,16}. Much of this conflict probably stems from the difficulty inherent in the detection of a small number of specific binding sites in the presence of a large number of nonspecific sites¹⁷.

This is especially true in view of the very "sticky" nature of the receptor-oestrogen complex after exposure to elevated temperatures (20–30 °C) at physiological salt concentrations¹⁸. The dissociation constant (K_d) for the binding of the receptor-oestrogen complex to various types of nuclei and non-biological surfaces, including glass, is 10^{-9} M at 25 °C (ref. 18). Thus, studies of the binding of the receptor-oestrogen complex to nuclei, chromatin and/or DNA in cell-free systems are susceptible to the error introduced by the masking effect of nonspecific binding.

Although the demonstration of nuclear acceptor binding is difficult *in vitro*, we have shown that a limited number of nuclear binding sites are involved in the production of maximal uterine growth^{4,5,19}. We have also demonstrated that the long term retention of 1,000–3,000 receptor-oestrogen complexes per cell is a requirement of uterine growth^{19,20}. Since uterine cells contain 15,000–20,000 receptor sites in the cytoplasm, it is apparent that only a fraction of these are required for maximal growth responses. These concepts and experiments are summarised in Fig. 1. We have suggested that the long term retention of the receptor-oestrogen complex in the nucleus is due to the binding of these complexes to a limited number of nuclear acceptor sites and that retention at these acceptor sites for more than 4–6 h is a requirement for true growth^{5,21}.

injected with either a small dose (0.1 µg) or a large dose (2.5 µg) of oestradiol-17β. The animals were killed and their uteri removed 1 or 6 h later. A nuclear pellet was prepared by centrifugation of a uterine homogenate (30–50 mg ml⁻¹) at 800g for 10 min²⁵. Although purified nuclei were not used, they behave in a fashion similar to that of this crude nuclear preparation (to be published elsewhere). The nuclear fraction was washed twice with cold TE buffer (0.01 M Tris, 0.001 M EDTA, pH 7.2, 4 °C). Salt extraction was performed by adding various concentrations of TK buffer (0.01 M Tris and 0.1–0.6 M KCl, pH 8.0, 4 °C) to the nuclear pellet with the mass to volume ratio maintained at 30–50 mg ml⁻¹. The nuclear pellets were resuspended in TK buffer and allowed to stand on ice, with mixing every 5–10 min, for 30 min. Nuclear fractions were centrifuged at 800g for 10 min and the KCl extract was decanted. TE buffer was added to the nuclear pellet (30–50 mg ml⁻¹) and the pellet was resuspended. If necessary, re-suspension was accomplished by rehomogenisation of the pellet with a glass-Teflon homogeniser. The nuclear suspension was then added to small assay tubes (5–10 mg per tube) and the quantity of receptor in the nuclear pellet was measured by ³H-oestradiol exchange²⁵.

The uterine nuclear fraction from rats that received 2.5 µg of oestradiol 1 h before they were killed contains a

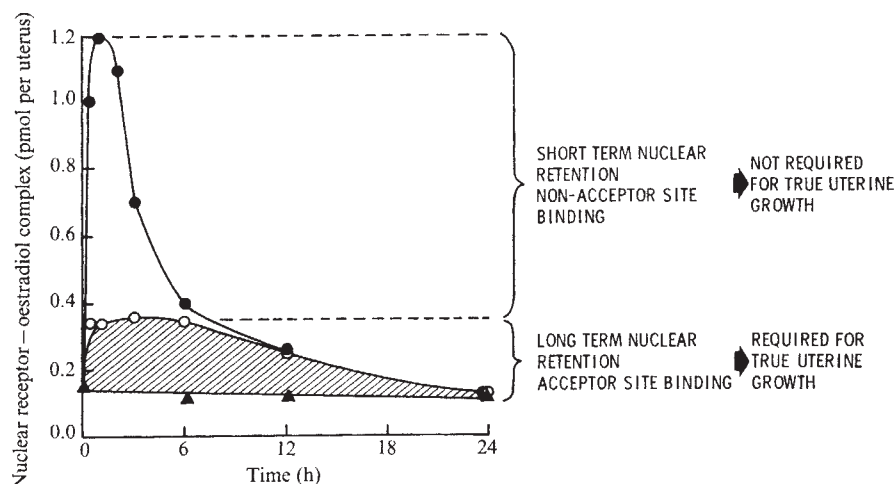


Fig. 1 Relationship between nuclear retention of the oestrogen receptor and uterine growth. Immature rats, 21–23 d old, were injected with either 0.1 or 2.5 µg of oestradiol and the accumulation and retention of the oestrogen receptor by the uterine nuclear fraction was examined by the ³H-oestradiol exchange assay^{4,25}. Uterine growth responses (DNA, RNA and protein content, wet and dry weight) were measured 24–48 h after injection (data not shown, see refs 4, 5, 19 and 20), and were stimulated maximally by 0.1 µg of oestradiol^{4,5,19,20}. Therefore we conclude that 0.1–0.2 pmol of receptor per uterus is required for the stimulation of true uterine growth²⁰. ●, 2.5 µg of oestradiol; ○, 0.1 µg of oestradiol; ▲, saline.

To test this hypothesis further we have used salt extraction of uterine nuclei to examine for differential extractability of the receptor-oestrogen complex. The rationale for the use of this technique was based on the observation, by several investigators, that extraction of nuclei with 0.3–0.4 M KCl does not remove all the nuclear-bound oestrogen^{22–24}, suggesting that some receptor-hormone complexes are bound more tightly than others. Immature rats 21–23 d old were

large number of receptor sites which are extractable with KCl concentrations less than 0.4 M (Fig. 2a). The quantity of receptor which remains in the nuclear fraction after exposure to KCl concentrations of 0.4 M or greater is approximately 0.1 pmol per uterus or 1,400 sites per cell. Thus, this small number of KCl-resistant receptor sites could represent those receptor sites which exhibit long term nuclear retention (Fig. 1). If this were true, the number of

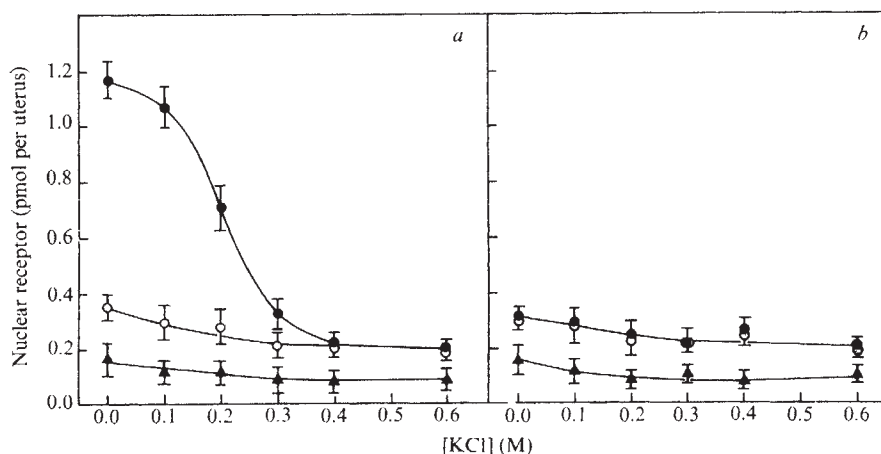


Fig. 2 Differential salt extraction of nuclear-bound oestrogen receptor. Immature rats were injected with oestradiol as described for Fig. 1. Uterine nuclear fractions were prepared 1 and 6 h after injection and differential KCl extraction was performed as described in the text. After extraction, the quantity of receptor which remained in the nuclear fraction was measured by the ³H-oestradiol exchange assay²⁵. a, 1 h; b, 6 h. Symbols as for Fig. 1.

KCl-resistant sites in animals receiving the large dose of hormone should equal the number receiving the small dose, since both doses produce the same number of long term retention sites (Fig. 1). As Fig. 2a shows, this seems to be the case. Likewise the number of KCl-resistant sites should be equal 6 h after injection of either dose of oestrogen and should be approximately the same as the number 1 h after the small dose. This also is the case, as Fig 2b shows. Therefore, we conclude that the number of receptor sites which remain in the nucleus after 0.4 M KCl extractions, are correlated with the number of sites which exhibit long term nuclear retention. The differential extraction of the receptor-oestrogen complex by KCl may reflect the binding of these complexes to different types of nuclear sites. Most (80-90%) of these nuclear receptor binding sites are of low affinity and extractable with KCl, whereas a minority (10-20%) are of high affinity and are non-extractable sites.

Other observations have suggested that receptor-hormone complexes manifest different characteristics once they have undergone nuclear binding. The rate of dissociation of the receptor-oestrogen complex is much slower when bound to chromatin than when free^{26,27}. DeHertogh has observed a small pool of nuclear bound receptor *in vivo* which exhibits a relatively slow rate of oestradiol exchange when compared with the majority of receptor²³. We have also observed two rates of exchange of the receptor-oestrogen complex when it is bound to the nucleus (unpublished). The exact relationship of these various observations to those reported here are not clear but they may reflect conformational changes in receptor-oestrogen complexes attendant on their association with specific nuclear acceptor sites. Such conformational alterations might result in associations of great affinity between receptor-hormone complexes and acceptor sites, thereby accounting for their resistance to KCl extraction. Since the number of these KCl-resistant nuclear sites is identical to that number of sites required for maximal uterine growth, we propose that these binding sites in the nucleus represent specific acceptor sites.

This work was supported by grants from the National Institutes of Health and the American Cancer Society. We thank K. O'Connor and J. Kovar for technical assistance.

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Received November 24, 1975; accepted March 2, 1976.

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Interferon action may not be effected by induction of a cellular ribonuclease

TREATMENT of animal cells with interferon or polyinosinic-polycytidylic acid (pI:pC) can prevent multiplication of animal viruses. In the case of most of such viruses the interferon action consists of an inhibition of the viral translation. For some viruses, such as the DNA-containing Simian virus 40 (SV40), there is evidence for direct inhibition both of transcription¹⁻³ and translation^{4,5}. Marcus *et al.*⁶ have shown, in chick embryo fibroblasts (CEF), that interferon or pI:pC are able to induce membrane-bound alkaline RNase activity. It was therefore proposed that degradation of viral RNA by membrane-associated RNase is indicative of a unifying concept of interferon action⁶. We attempted to determine whether the induction of interferon is paralleled by an induction of membrane-associated RNase activity in CEF and in primary African green monkey kidney (AGMK) cells. Our results show RNase induction in CEF but not in AGMK cells.

We have found a relatively high level of RNase associated with the membranes from unstimulated CEF compared with membranes from AGMK cells (see Table 1 and Fig. 1). Previous stimulation of CEF with pI:pC resulted in a sevenfold increase in RNase level. This level in the assay mixture (0.25 ml), containing 4 μ g membrane protein, is equivalent to the activity of 30 ng ml⁻¹ bovine RNase. These data obtained using CEF agree well with the results of Marcus *et al.*⁶. Stimulation of AGMK cells with pI:pC, however, failed to induce a significant increase in RNase level in those cells. Similar results with both CEF and AGMK cells were obtained when membranes were prepared according to the method of Joklik *et al.*⁸.

The RNase induced in CEF also proved to be active using host-specific RNA as a substrate, indicating that the RNase does not show a specificity for viral RNA; this might be expected if it were involved in the action of interferon. We are, therefore, able to confirm the findings of Marcus *et al.*⁶ for CEF, but we cannot support the

Table 1 Induction of interferon and isolation of membranes from CEF and AGMK cells

Interferon induction*		Membrane isolation	
Cell type and DEAE-dextran treatment ($\mu\text{g ml}^{-1}$)	pI : pC treatment ($\mu\text{g ml}^{-1}$)	Total membrane protein in 10^8 cells† (μg)	Membrane protein in 0.25 ml of RNase assay (μg)
AGMK	0	440	14.8
500	20	340	15.0
CEF	0	270	4.0
10	2	285	4.2

Membranes were prepared in discontinuous sucrose gradients^{6,7}. Seven unequal membrane bands were visible. Band 3 (from top) consisting of a layer of smooth membranes, and band 4, containing rough and smooth membranes⁷, were combined. These fractions were subsequently subjected to RNase assay. Substrates in tests¹ were ³H-uridine-labelled AGMK cell RNA and *in vitro* transcribed ³H-SV40 cRNA.

* Cells were treated with the mixture of DEAE-dextran and pI:pC for a 15-min period 18 h before membrane isolation³. This treatment renders more than 99% AGMK cells resistant to an infection with SV40 (multiplicity of infection = 10) and renders CEF resistant to infection with vesicular stomatitis virus (multiplicity of infection = 1) for a period of 24-48 h.

† Gradient fractions 3 and 4 (ref. 7) were combined.

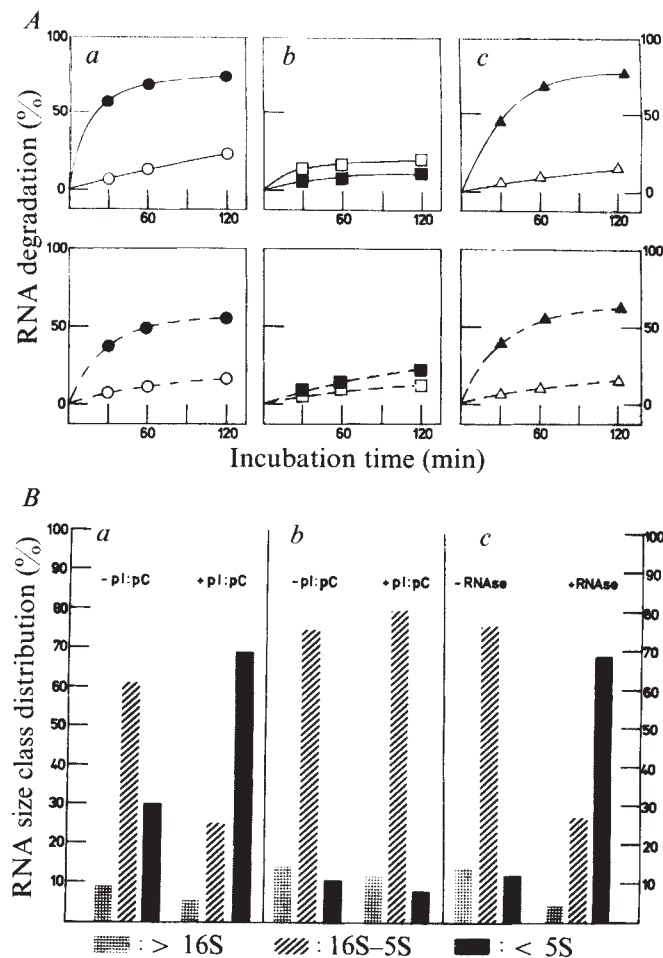


Fig. 1 A, Degradation of ³H-RNA after incubation with membrane fractions from a, CEF (○, ●) and b, AGMK cells (□, ■) pretreated with pI : pC (closed symbols) and untreated (open symbols). Assay mixture contained (total volume 0.25 ml) 0.1 ml membrane protein preparation (Table 1), 0.1 M Tris-HCl (pH 8.2), 7.5 mM Mg²⁺, 1 mM EDTA, and 2.5 × 10⁹ c.p.m. ³H-RNA substrate. Incubation temperature was 37 °C. Substrate was ³H-labelled SV40 cRNA⁹ (specific activity 1 × 10⁴ c.p.m. μg⁻¹; —); and ³H-RNA from ³H-uridine-labelled AGMK cell RNA (specific activity 1.5 × 10⁴ c.p.m. μg⁻¹; - - - -). Degradation of ³H-RNA during incubation was assayed by scintillation counting of TCA precipitates on Whatman GF/C filters. c, Controls: incubation with 30 ng ml⁻¹ bovine RNase (EC 3.1.4.22; ▲) and without RNase addition (△). B, Distribution of three ³H-SV40 cRNA size classes after 120-min incubation period with membrane protein (Table 1) from pI : pC-treated (+pI : pC) and untreated (-pI : pC) CEF (a) and AGMK cells (b). c, Controls: incubation with bovine RNase as above. Size classes of ³H-SV40 cRNA were determined by centrifugation in formaldehyde-sucrose gradients⁹.

Changes in histone f2a2 associated with proliferation of Friend leukaemic cells

HISTONE-HISTONE interactions with DNA form the basic structural unit of chromatin¹⁻³, and quantitative changes in the cellular histones correlate with alterations in chromatin structure^{4,5}. More subtle changes in chromatin structure follow chemical modification of the histones. These changes may enable genes to assume proper configuration for specific regulation by the non-histone proteins.

Growth of Friend cells *in vitro* with 1-2% dimethylsulphoxide (DMSO) induces globin mRNA synthesis^{6,7}, the appearance of erythrocyte membrane antigen⁸, an increase in haem synthesis and iron uptake⁸, an alteration in purine metabolism⁹ and morphological changes characteristic of erythroid differentiation¹⁰. For this response the cells must complete S phase in the presence of 1-2% internal concentration of DMSO¹¹. Evidence suggests that DMSO or a metabolic product acts at the level of the chromatin, causing biochemical and physical alteration in DNA-protein interactions¹²⁻¹⁴. If this is true, perhaps the ability of the cell to respond to DMSO depends on a particular chromatin structure. We have established DMSO-inducible and DMSO-non-inducible cell lines *in vitro* from subcutaneous tumours derived from a Friend leukaemic spleen in DBA/2J mice. Analysis of the histones from these cell lines has demonstrated a striking difference in histone f2a2 during establishment of the lines in culture, especially when DMSO-responsive and unresponsive cells are compared.

In acid-urea gels¹⁵, no difference in the kinds or amounts of histones from the different cell types was found. Separation on acid-urea Triton gels^{16,17}, however, resulted in a finer resolution of the histones, and demonstrated a marked difference in the f2a2 histone subfractions from different Friend leukaemic cells (Table 1). In these gels, histone f2a2 from normal spleen and liver tissue separated into two fractions, f2a2₁ and f2a2₂, in a ratio of 3 : 1 (Table 1). A similar f2a2 ratio was found for leukaemic spleen tissue or subcutaneous tumour cells taken from the mouse. Friend tumour cells established as *in vitro* cell lines (for example, TP46, TP63), however, showed an increase in f2a2₂ and a f2a2₁ : f2a2₂ ratio of about 2 : 1. These freshly established cell lines were unresponsive to DMSO (Table 1). After several months' passage in culture, these cell lines began to show a response to DMSO with synthesis of haemoglobin. Coincident with this change in response to DMSO was a further increase in f2a2₂ with the ratio of f2a2₁ : f2a2₂ now approaching 1 : 1 (Fig. 1, Table 1). Less extensive differences were observed in f2b and f3 histones. The f2a2 ratio was not altered when DMSO-responsive or unresponsive cells were grown subcutaneously into a tumour in a mouse (C17, Table 1). Furthermore, in contrast to the gradual adaptation of Friend tumour cells to tissue culture, these tumour cells derived from tissue culture, with increased f2a2₂, adapted immediately to growth *in vitro* and retained their original response to DMSO.

generalisation drawn from this phenomenon that resulted in the unifying concept of interferon action.

An inhibition by interferon of SV40 tumour antigen formation in monkey cells microinjected with *in vitro* transcribed SV40-specific RNA has been found^{4,5}. In terms of this inhibition, the failure of pI : pC to induce in AGMK cells a membrane-associated RNase activity, suggests that inhibition of translation is unlikely to be affected by induction of membrane-associated RNase. It has also been found that interferon treatment is also ineffective in inducing RNase activity in embryonic hamster cells. P. I. Marcus (personal communication) has obtained the same results with cells from quails and ducks.

We thank Miss Erika Koch for assistance and Dr H. Werchau for advice in preparing membranes. Supported by the Deutsche Forschungsgemeinschaft.

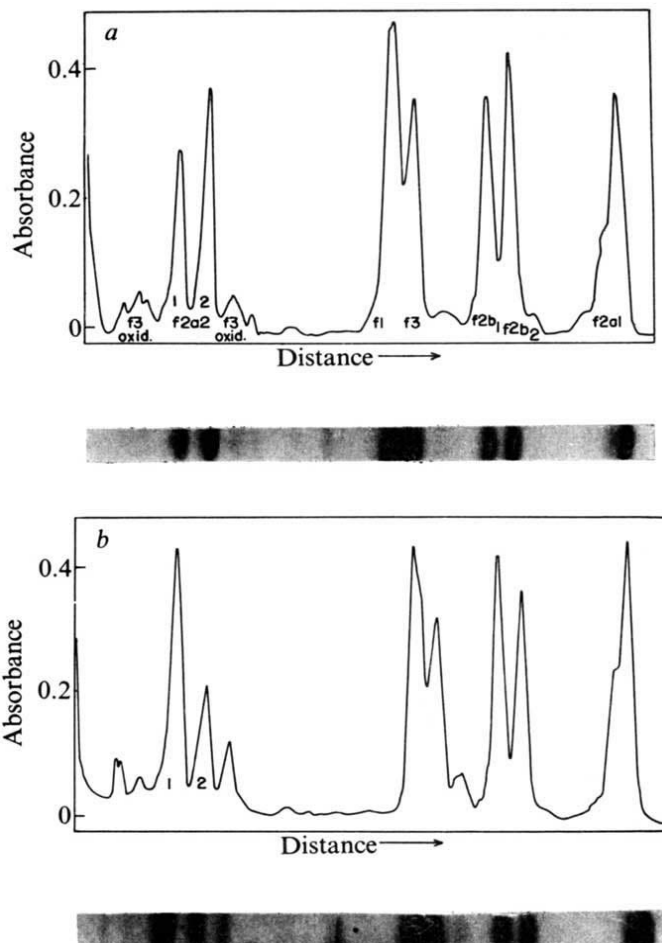
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Fig. 1 Histones from C17 cloned cell line (a), and leukaemic spleens (b). Tissue culture cell lines were obtained from tumours initiated by placing a piece of Friend leukaemic spleen subcutaneously in a mouse. Cells were grown in basal media, Earle's salts (Gibco) containing penicillin and streptomycin and 15% foetal calf serum. For isolation of nuclei and histone extraction, we used the following procedure. Cells collected by centrifugation were resuspended at 10^8 cells per ml in buffer 1 (0.05 M Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 0.02 M KCl, 0.25 M sucrose, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 5 mM sodium bisulphite) and incubated at 4 °C for 5 min before being sedimented by centrifugation (5 min at 2,500 r.p.m. in a SS34 rotor (Sorvall)). This procedure was repeated. Cells were then suspended in 10 volumes of buffer 2 (5 mM Tris, pH 7.1, 5 mM $MgCl_2$, 0.05 mM dithiothreitol, 0.1 mM PMSF) and Triton X-100 was added to a final concentration of 1%. After 10 min, the nuclei were sedimented by centrifugation at 2,500 r.p.m. for 10 min (SS34 rotor), washed in buffer 2 without Triton and suspended in 2.2 M sucrose, 1 mM $MgCl_2$, 0.1 mM dithiothreitol and 1 mM PMSF. Centrifugation at 40,000g for 1 h in the SW27 (Beckman) sedimented the clean nuclei to the bottom of the centrifuge tube. The purified nuclei were washed twice with 0.14 M NaCl, 0.01 M Tris at pH 7.5 before extracting them three times for 1 h each with 0.4 N H_2SO_4 at 4 °C. The histones were precipitated from the combined supernatants by addition of 5 volumes of 95% ethanol and placed at -20 °C overnight. Each histone class in the Triton gels was identified by two methods. (1) Individual histone fractions were extracted by a selective chemical extraction described by Johns²². The purity of the fractions was checked by their separations on acid-urea gels¹⁵. The same individual histone samples were then separated on the Triton-acid-urea gels and their migration was identified. (2) Large diameter Triton gels were prepared on which 1 mg of protein was separated. The gels were sliced into 2-mm sections from which the histones were extracted overnight in 0.15 M NaCl. The location of each histone was determined by assaying each fraction with individual anti-histone antisera in the complement fixation assay described by Stollar and Ward²³. For this figure, 25 µg of histone was separated on 12% polyacrylamide gel containing 8 M urea and 6 mM Triton X-100^{16,17}. Bands 1 and 2 are f2a2₁ and f2a2₂, respectively. The gels were stained with 0.4% Amido Black and acetic acid : methanol : water (1 : 5 : 14) and scanned at 570 nm with a spectrophotometer (Beckman).



Many different DMSO-responsive cell lines of Friend leukaemia, including the original 745 line from C. Friend, have this altered f2a2. The change in the ratio of the f2a2 subfractions seems to be a prerequisite for the ability of the cells to respond to DMSO. The f2a2 ratio does not change when the cells respond to DMSO.

With increasing time in culture, previously unresponsive cell lines began to respond to DMSO and to show an increase in f2a2₂ and concomitant decrease in f2a2₁. An uncloned cell line, TP46, established from passage 46 of a leukaemic subcutaneous tumour, was unresponsive to DMSO and retained an f2a2₁ : f2a2₂ ratio of 2 : 1 when examined at passages 5, 10 and 18. At passage

36, 10 weeks later, however, the cell line responded to 1.5% DMSO and demonstrated an increase in f2a2₂ (Fig. 2). Four cloned cell lines, C7A→D, showing different relative responses to DMSO, were derived from another cloned cell line, C7, established from passage 25 of uncloned line TP32. C7 was initially unresponsive to DMSO but began to show a response after several months' passage in tissue culture. Clone C7C did not synthesise detectable levels of haemoglobin when cultured in 1–2% DMSO. In contrast, C7D responded well to small amounts of DMSO. C7A and C7B were intermediate in their response. Morphologically, all cell lines were similar. Analysis of the histones in these cell lines showed a direct correlation

Table 1 Percentage of each f2a2 subfraction in the total f2a2

	DMSO response	% f2a2 Histone in total histone	% Subfractions in total f2a2		
			f2a2 ₁	f2a2 ₂	f2a2 ₁ /f2a2 ₂
Normal spleen cells		22	75	25	3.0
Normal liver cells		18	75	25	3.0
Phenylhydrazine-treated mouse spleen cells		21	78	22	3.5
21-d Leukaemic spleen cells		17	77	23	3.3
Tumour cells (passage 40, <i>in vivo</i>)		21	77	23	3.3
Uncloned cell lines					
TP46P18	—	18	70	30	2.3
TP46P39	+++	22	50	50	1.0
TP63P8	—	17	66	34	1.9
Cloned cell lines					
C7A p8	++	20	60	40	1.4
C7B p8	++	21	63	37	1.6
C7C p7	+	17	66	34	1.9
C7C p27	++	20	62	38	1.5
C7D p8	++++	21	47	53	0.8
F1* p50	+++	20	55	45	1.2
C17 p10	++++	19	44	56	0.8
Tumour from C17 cells	++++	19	47	53	0.9

Histones were separated on 12% polyacrylamide gels containing 8 M urea and 6 mM Triton X-100. The percentage of each f2a2 subfraction was determined by weighing photocopy paper of each histone peak from a densitometer tracing and dividing that by the combined weight of both subfractions. The amount of bound dye was directly proportional to the amount of protein in each band (< 20 µg) (ref. 20). TP, tumour passage; +, relative amount of haemoglobin produced in response to DMSO.

* Original line 745.

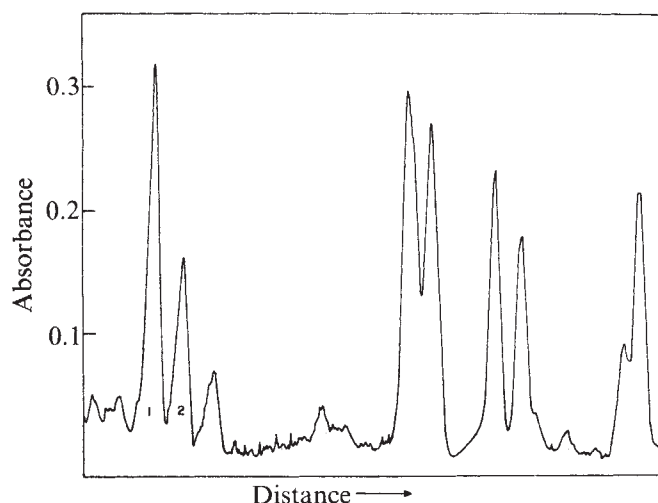


Fig. 2 Histones from uncloned cell line (TP46P18) were extracted and separated as described in the legend to Fig. 1.

between the amount of haemoglobin synthesised in response to DMSO and the amount of f2a₂ (Fig. 3). The results from C7A and C7B may represent an average from responsive and unresponsive cells now present in these originally cloned lines or a decreased DMSO response in all cells. The fact that both types of cells were cloned from the original cloned C7 lines suggests that with time in tissue culture a change towards inducibility occurs in some, but not all, cells.

f2a₂ Histone is a major component of chromatin representing 20% of the histones of all mouse cells tested including both inducible and non-inducible cell lines. The changes observed in f2a₂ subfractions are suggestive of either (1) a chemical modification of the f2a₂ fraction causing it to migrate in a different position in the gel, that is, f2a₂, or (2) a change in the synthesis of the two f2a₂ subtypes similar to the situation found during sea urchin development¹⁸. The strong association of this histone change with the ability of the cell to respond to DMSO suggests that the change opens up groups of genes which can now be induced by DMSO. Evidence from different laboratories indicates that DMSO affects both physical structure and biochemical function of chromatin^{12,13}. In the Friend cell system, DMSO produces a group of changes related to erythroid maturation⁶⁻¹⁰. This pleiotropic response may be explained by (1) a structural change in the chromatin of the already committed erythroid cell produced by the f2a₂ modification and (2) a further change in this altered chromatin by DMSO.

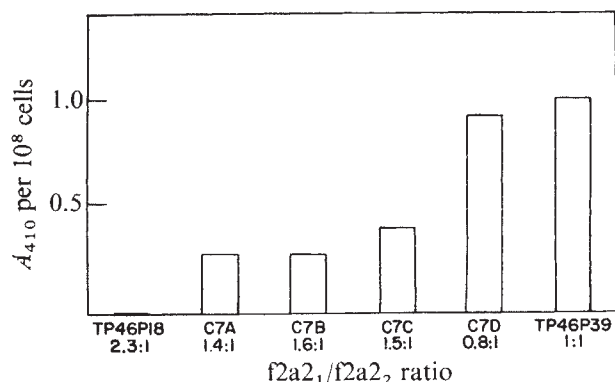


Fig. 3 Relationship of haemoglobin production to relative amounts of f2a₂. After 5 d in 1.5% DMSO, cells were counted, washed twice in 0.14 M NaCl and separated into two fractions. One fraction was placed in distilled water and the cells were disrupted in 1% sodium dodecyl sulphate. Cell debris was removed by centrifugation at 1,500g for 10 min and the supernatant solution was read at 410 nm (refs 20 and 21). From the second fraction the histones were extracted (Fig. 1) and the amount of each f2a₂ subfraction was determined.

Histones extracted from leukaemic spleens and erythroid spleens do not show the increase in f2a₂ seen in DMSO-responsive Friend cell lines, although haemoglobin synthesis occurs. This may reflect the heterogeneity in the cells taken from these spleens. Studies of early erythroid cells isolated from these tissues are in progress to resolve this question. Whatever differences may exist between the control of erythroid development *in vitro* and *in vivo* seem to be circumvented by DMSO in the presence of an alteration of f2a₂, since the contribution of two factors leads to apparently normal erythroid maturation⁶⁻¹⁰.

The fact that histone f2a₂ is so conserved during evolution suggests that the specific change we observe in the ratio of the f2a₂ subfractions serves a purpose that enables the Friend cells to grow autonomously in tissue culture and to respond to DMSO *in vitro*.

We would like to thank D. B. Stollar, of Tufts Medical School, for his assistance with the complement fixation assay and L. Cohen and C. Alfagime, of the Institute for Cancer Research, for their helpful discussions.

This work was supported in part by a grant from the National Leukemia Association, a fellowship of the Leukemia Society of America (L.A.B.) and a research career development award (S.B.L.).

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Received November 28, 1975; accepted February 19, 1976.

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DNA ligases during rat liver regeneration

DNA ligase catalyses the formation of phosphodiester bonds at single-strand breaks in double-stranded DNA¹. Since DNA is replicated discontinuously, and radiation-induced DNA single-strand breaks are effectively repaired in both bacteria and eukaryotes, bacteria and mammalian cells will require DNA ligase function both for replication and repair of DNA²⁻⁵. A DNA ligase activity has also been found in and purified from mammalian cell extracts⁶. It required ATP as a cofactor and was found in highest amounts in tissues containing rapidly dividing cells. In agreement with these data it has been reported that the DNA ligase activity increased five- to tenfold in rat liver extracts during liver regeneration^{7,8}. We have shown that mammalian cells contain two different DNA ligases (I and II) both of which are found in nuclei and cytoplasm, but neither of which is predominantly a mitochondrial enzyme^{9,10}. Both enzymes require ATP but have different molecular weights, fractionation properties, and heat stability. Furthermore, rabbit antibodies against purified calf thymus DNA ligase I

effectively inhibit DNA ligase I from calf, mouse, rabbit, and human tissues, whereas DNA ligase II from the same sources is not inhibited. It was therefore of interest to determine whether the increase in total DNA ligase activity reported to occur during liver regeneration was due to an increase in both DNA ligase I and DNA ligase II activities or due to an increase in only one of the two enzymes. Such determinations are now possible when chromatographic properties of the enzymes are known, and antibodies against DNA ligase I are available. This report shows that the level of DNA ligase I activity increases about 15-fold during rat liver regeneration, whereas the level of DNA ligase II activity remains essentially unchanged.

Male Sprague-Dawley rats (~200 g) were partially hepatectomised (to about 70%) as described previously¹¹. At indicated times after hepatectomy two rats were killed, the livers immediately taken out, rinsed in ice-cold 0.25 M sucrose, rapidly frozen to -70°C , and kept at this temperature until preparation. Cell extracts from equal amounts of liver from two rats (approximately two half livers) were prepared and purified by streptomycin treatment, ammonium sulphate fractionation (40–65% saturation), and stepwise phosphocellulose chromatography as described previously¹⁰. These fractionation steps were used to remove nucleases and other activities potentially interfering in the enzyme assays. Earlier experiments with calf tissue DNA ligases have not given indications that any of the two DNA ligases are selectively lost during these purification steps¹⁰. DNA ligases I and II were separated by hydroxyapatite chromatography: I activity was eluted with 0.14 M potassium phosphate (pH 7.2) and II with 0.40 M potassium phosphate¹⁰. Assignment

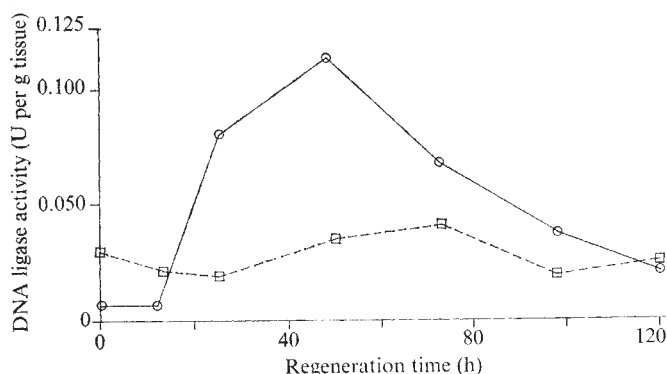


Fig. 1 Levels of activity of DNA ligases I (○) and II (□) during rat liver regeneration. Each point represents duplicate experiments with several different enzyme concentrations.

of these fractions with DNA ligase I and II were verified by experiments with the anti-serum against calf thymus DNA ligase I, which inhibited more than 95% of the ligase activity in the 0.14 M eluate, whereas the activity in the 0.40-M eluate was inhibited 0–20% due to tailing of DNA ligase I during chromatography, as described previously¹⁰. The eluted hydroxyapatite fractions were pooled, concentrated by ammonium sulphate precipitation, redissolved, and dialysed against 0.3 M NaCl–0.05 M Tris-HCl–1 mM EDTA–1 mM dithiothreitol (pH 7.4) for 14–16 h before assay. DNA ligase activity was determined after preincubation of the fractions at 0°C for 5 min with either control serum or antiserum against DNA ligase I. The ligase assay used measures the conversion of $5'$ - ^{32}P -phosphoryl termini at single-strand breaks in double-stranded DNA to a form resistant to alkaline phosphatase^{10,12}.

The data obtained for the different time points are given in Fig. 1. Normal rat livers contain a relatively low level of DNA ligase activity, and most of this activity is due to DNA ligase II. The levels of this enzyme seem to remain essentially constant during liver regeneration as the measured variation is less than twofold. In contrast, DNA ligase I activity increases approximately 15-fold to a broad maximum at about 48 h regeneration time, and is thus the dominant DNA ligase activity in extracts from regenerating rat livers. After 5 d regeneration, the level of DNA ligase I had almost returned to the value found in

normal rat liver. It is clear that the previously discovered increase in DNA ligase activity during liver regeneration^{7,8} is almost entirely due to an increase in DNA ligase I. Together with our earlier published results^{9,10}, the present data strongly indicate that mammalian cells contain two different DNA ligases.

The isolated hydroxyapatite fractions of DNA ligases I and II from the 48-h regenerating livers were used for two additional control experiments. When separately chromatographed on Sephadex G-150 in 0.3 M NaCl–0.05 M Tris-HCl–1 mM EDTA–1 mM dithiothreitol pH (7.4), the ligase activity of these fractions showed identical elution properties to the corresponding calf thymus enzymes. Furthermore, when the separate enzyme fractions were heated at 45°C for 5 min in the same buffer as that used for gel chromatography (protein concentration: 3 mg ml⁻¹), the rat DNA ligase I fraction retained more than 95% of its initial activity, whereas the rat DNA ligase II retained less than 5% of the initial activity. These data show that the rat DNA ligases are of similar heat lability and molecular weight as the corresponding calf thymus enzymes. To determine whether the apparent increase in DNA ligase I activity could be explained by the presence of DNA ligase I inhibitors in the 0-h liver fractions, or by the presence of effectors in the 48-h fractions, mixing experiments were carried out. Calf thymus DNA ligase I (1×10^{-3} U 1,000-fold purified enzyme) was separately mixed with several concentrations (0.5 – 3×10^{-4} U) of the 0-h or 48-h hydroxyapatite fractions of rat liver DNA ligase I. In all cases, the observed DNA ligase activity was identical ($\pm 10\%$) to the expected value calculated as the sum of the individual ligase activities of the mixed fractions. Thus, the 15-fold increment in DNA ligase activity during liver regeneration did not seem to be due to artefacts such as differences in interfering factors between the extracts. It is clear that whereas DNA ligase I activity in normal rat liver is low, the cells still contain detectable amounts of this enzyme. Similar control experiments with fractions containing DNA ligase II also demonstrated the absence of assay artefacts in this case. To check liver regeneration, the amount of DNA polymerase α activity was measured in the cell extracts, and was about five times higher after 24 h regeneration than in extracts from control livers, in agreement with earlier determinations¹³. Moreover, the weight of the livers successively increased during regeneration.

Regenerating liver has often been used as a system in which a population of non-dividing (G_0) cells is turned into an actively proliferating state with a high level of DNA replication. Many enzymes associated with synthesis of DNA and its precursors are known to increase during liver regeneration, but the time required for maximal induction varies considerably for the different enzymes. Moreover, in the case of multiple enzymes with similar function, the response of the individual enzyme activities to regeneration might be quite different. In the case of mammalian DNA polymerases, the DNA polymerase α activity shows a five- to sevenfold increase during rat liver regeneration¹³, and DNA polymerase γ activity has also been reported to increase in cells actively synthesising DNA¹⁴. In contrast, the level of DNA polymerase β activity remains constant or shows only a minor increase in the same conditions^{13,14}. The increase in DNA polymerase α and γ during cell proliferation in these and other systems has been considered as circumstantial evidence for their involvement in DNA replication^{13,14}. The present data on DNA ligases I and II levels during liver regeneration clearly resemble the previous findings for the DNA polymerases. In this regard, note that DNA ligase I has also been found in relatively large amounts in rapidly dividing cell populations such as calf thymus and mouse ascites tumour cells, but in much lower amounts in calf liver; whereas DNA ligase II levels have shown relatively minor variations between different tissues¹⁰. As the levels of both DNA polymerase α and DNA polymerase γ activities increase during cell proliferation, it seems possible that DNA ligase I acts together with one or both of these enzymes in DNA replication, and that the

cellular levels of that polymerase and the ligase are regulated in coordination. DNA polymerase β and DNA ligase II, neither of which show increased levels during cell proliferation, may then have functions in maintaining the integrity of DNA.

I thank Dr Tomas Lindahl for discussions, Dr Bernd Otto for carrying out the measurements of DNA polymerase α activity, and Ms Berit Sperens for assistance. This work was supported by the Swedish Natural Science Research Council, the Swedish Cancer Society and the Karolinska Institutet.

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Received December 23, 1975; accepted February 9, 1976.

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Single-stranded regions in regenerating rat liver DNA

SEVERAL groups have identified single-stranded regions in actively replicating eukaryote DNA^{1–5}. We have used isopycnic caesium sulphate gradients containing silver ion, and a single-strand-specific endonuclease to determine the single strandedness of DNA from actively regenerating rat liver, and from 'control' liver of partially hepatectomised rats that had been allowed to regenerate their normal liver mass. More single-stranded DNA was observed in regenerating liver than in control livers.

One-month-old male Sprague-Dawley rats were subjected to partial hepatectomy⁶. Seven animals were killed 20.5 h later and their livers were removed. A second group of seven was housed and fed *ad libitum* for 14 d before being killed; in these conditions more than 95% of the original liver mass was restored⁷. Nuclei were isolated by the method of Blobel and Potter⁸ and DNA was purified using Marmur's procedure⁹.

Figure 1 shows the results of $\text{Cs}_2\text{SO}_4\text{-Ag}^+$ isopycnic centrifugation of DNA from the two groups of hepatectomised rats. The gradient profile of a mixture of native and heat-denatured 14-d hepatectomised rat DNA is given in Fig. 1a. Denaturation of DNA causes an increase in silver ion binding leading to an increase in buoyant density¹⁰. Denatured DNA banded with a peak density of 1.63 g cm⁻³ and native DNA banded with a peak density of 1.50 g cm⁻³. The identities of the peaks were confirmed when native DNA was centrifuged separately (data not shown). When DNA from actively regenerating (20.5-h) liver was centrifuged in identical conditions (Fig. 1b), its buoyant density was greater than that of 14-d regenerating DNA by 0.09 g cm⁻³ (Fig. 1a). After dialysis against 0.015 M NaCl–0.0015 M sodium citrate, pH 7.5, the peak fraction of Fig. 1b had a characteristically double-stranded thermal denaturation pattern and a hyperchromicity of 37%. (It should be noted, however, that thermal denaturation patterns detect only large amounts of single-strandedness.) Thus, DNA from actively proliferating liver seems to be more single-stranded than DNA from control liver.

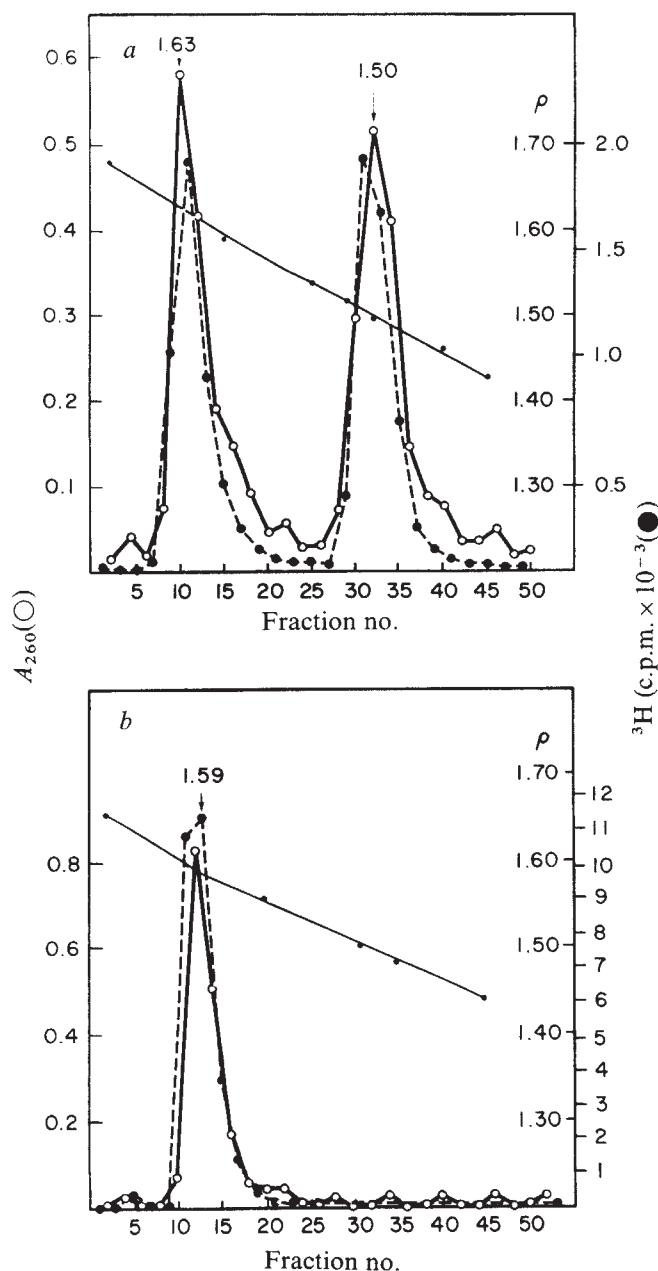


Fig. 1 $\text{Cs}_2\text{SO}_4\text{-Ag}^+$ isopycnic centrifugation of 20.5-h and 14-d regenerating rat liver DNA. The procedure has been described before¹⁰. Rats were injected 20 h after hepatectomy, through the tail vein, with ^3H -methyl thymidine (New England Nuclear, 50.8 Ci mmol⁻¹) at a concentration of 1 μCi per body weight. Specific activities of the 20.5-h and 14-d hepatectomised rat liver DNAs were 780 c.p.m. μg^{-1} and 127 c.p.m. μg^{-1} , respectively. DNA was dialysed sequentially against 0.1 M EDTA, pH 8, 0.1 M NaClO₄ and 0.01 M Na₂SO₄. The DNA (200 μg) was added to 6.3 g Cs_2SO_4 (Gallard-Schlesinger ultrapure) in sodium borate buffer, pH 9.2, to give a final concentration of 5 mM. AgClO_4 was added in drops to a final concentration of 16 μM and a molar ratio of $\text{Ag}^+/\text{DNA-P}$ of 0.27. The solution was adjusted to a density of 1.5 g cm⁻³ and a final volume of 10 ml by the addition of water and/or Cs_2SO_4 . Centrifugation was performed in a Beckman model L3-50 ultracentrifuge in a type 65 rotor at 20 °C for 70 h at 35,000 r.p.m. Fractions were collected from the bottom. Densities of samples of selected fractions were calculated from refractive indices determined with a Bausch and Lomb refractometer. Samples (0.1 ml) of odd-numbered fractions were applied to filter disks, dried, and washed sequentially in cold 5% trichloroacetic acid, ethanol, and ether. The radioactivity was determined in a Liquefluor-toluene cocktail with a Beckman LS-355 liquid scintillation counter at an efficiency of 54% for tritium. Even-numbered fractions were diluted with 0.5 ml of water and the A_{260} was determined with a Gilford model 2400-2 spectrophotometer. *a*, 100 μg heat-denatured DNA plus 100 μg native DNA from 14-d regenerating rat livers. *b*, 200 μg native 20.5-h regenerating rat liver DNA.

The kinetics of release of acid-soluble material from single-stranded DNA by S1 nuclease from *Aspergillus oryzae*¹¹ was determined using heat-denatured calf thymus DNA. After 30 min of treatment with S1 nuclease, approximately 90% of denatured DNA was rendered acid-soluble. To test for the presence of endogenous nucleases in our rat liver DNA preparations, the DNA was incubated without added enzyme. No significant release of acid-soluble material was detected.

To confirm that our S1 nuclease preparation was free of contaminating double-strand nuclease activity, a covalently closed circular DNA (R6K plasmid¹²) was examined by sedimentation in sucrose density gradients before and after treatment with S1 nuclease (data not shown). The only action observed was a slow nicking of the supercoiled covalently closed circular form. A similar slow nicking of another covalently closed circular molecule (ϕ X174 RFI) by S1 nuclease has been reported by Godson¹³. Thus, our nuclease seems to be free of double-strand nuclease activity.

DNA from each group of rats was treated with S1 nuclease. The quantities of A_{260} U of 20.5-h and 14-d post-hepatectomy DNAs that were sensitive to S1 nuclease were 3.9% and 1.3%, respectively. Thus, DNA obtained 20.5 h after hepatectomy contained three times more single-strand nuclease-sensitive sites than DNA from 14-d regenerate livers. Since the mitotic index of cells from actively regenerating liver is about 300 times higher than that of cells from normal liver¹⁴, our results seem to correlate with cellular replication. Our findings agree with those of Suzuki, *et al.*¹⁵, who, using the electron microscopic immunoperoxidase method, found more single-stranded sites in DNA in nuclei from regenerating liver than in nuclei from control liver. DNA from actively replicating cells of the iris of the newt, *Triturus*, has been reported to contain more single-stranded regions than DNA from non-replicating cells⁵.

In summary, there seem to be more single-stranded regions in DNA from actively dividing liver cells than in DNA from non-dividing liver cells. The physiological significance of single-stranded regions is not clear, but they are likely to be involved in DNA replication. This work was supported by grants from the National Cancer Institute and the American Cancer Society. We thank Dr Francis L. Macrina for R6K DNA.

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Received December 24, 1975; accepted February 24, 1976.

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obscure. There is no evidence to indicate that NAL can bind to DNA^{3,4}, and none of the enzymes known to be involved in DNA replication is affected by NAL *in vitro*³⁻⁶. But it is known that *nalA* mutants of *Escherichia coli* are resistant to the drug⁷, as are *in vitro* DNA replicative systems derived from such mutants³. This suggested that a comparative study of DNA replication in *nalA* mutants and otherwise isogenic bacteria should facilitate identification of the hitherto unknown NAL target. We report here that NAL specifically arrests the later stages of chromosomal replication in bacteria sensitive to the drug.

Continuous DNA-labelling with ³H-thymidine was done as before⁸ using *E. coli* strains KL16 (NAL^s) and MH5 (a *nalA* mutant of KL16; ref. 7); in the presence and absence of the drug at 20 μ g ml⁻¹. The results (Fig. 1) show that addition of NAL rapidly inhibited DNA synthesis in strain KL16 without significantly affecting that of strain MH5.

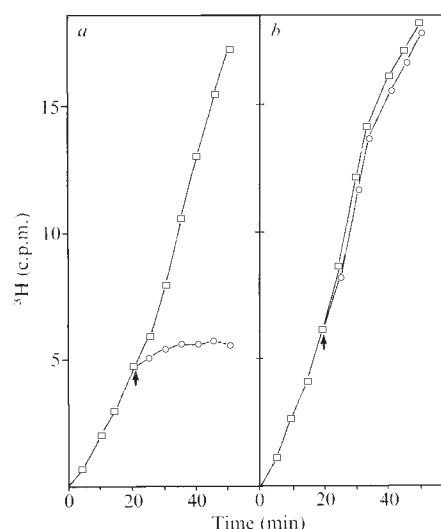


Fig. 1 Incorporation of ³H-thymidine into DNA measured by continuous labelling in the presence and absence of NAL at 20 μ g ml⁻¹. a, *E. coli* KL16; b, *E. coli* MH5. For both strains: $\square$, no drug; $\circ$, drug-treated. The arrows indicate the time of addition of NAL to 20 μ g ml⁻¹. Bacteria (initially 10⁷ viable units per ml) were cultured at 30 °C in Davis and Mingioli (DM) medium containing ³H-thymidine (0.1 μ Ci ml⁻¹) and deoxyadenosine (50 μ g ml⁻¹)⁸. At the times shown 1-ml samples were treated with 0.1 ml trichloroacetic acid at 0 °C and filtered through Whatman GF/C filters. After washing with 1 volume of 1% trichloroacetic acid, boiling distilled water (3 volumes), 5% NaCl (1 volume) and 5% acetic acid (2 volumes), the filters were dried and radioactivity was determined by scintillation counting.

Pulse labelling^{9,10}, however, yielded markedly different results. Using alkaline sucrose density gradient centrifugation with a 5-min pulse of ³H-thymidine and a 15-min label-free chase period, DNA replication in the two strains was examined both in the absence of the drug and after 20 min of treatment with NAL (20 μ g ml⁻¹). Monomeric linear ¹⁴C-labelled phage λ DNA was used as a sedimentation marker throughout. In the untreated cells of strain KL16 an even distribution of single-stranded DNA fragments was observed throughout the gradient after pulse-labelling (Fig. 2a). During a subsequent label-free chase period all the low and most intermediate sized DNA precursors had been converted into DNA of high molecular weight. When the bacteria were treated with NAL for 20 min before a pulse label, single-stranded DNA fragments were still synthesised (Fig. 2b). DNA of high molecular weight, however, was absent and there was an abnormal accumulation of DNA of intermediate size of about 38S. Moreover, these 38S DNA precursors could not be chased into DNA of higher molecular weight.

When the amount of pulse label incorporated into DNA

Nalidixic acid and bacterial chromosome replication

NALIDIXIC acid (NAL) has been described as a specific inhibitor of bacterial DNA synthesis *in vivo* and *in vitro*¹⁻³, but its mechanism of action on susceptible bacteria remains

Fig. 2 DNA size distributions. *a*, Distribution of pulse-labelled (●) DNA from strain KL16 obtained by alkaline sucrose gradient centrifugation; ○, results obtained after a similar pulse followed by a 15 min label-free chase period. *b*, Distribution of pulse-labelled (●) DNA from strain KL16 obtained by alkaline sucrose gradient centrifugation of a culture treated with NAL ($20 \mu\text{g ml}^{-1}$) for 20 min before isolation; ○, results obtained after a similar pulse followed by a 15-min label-free chase period in the presence of NAL. (Fractions are numbered from the top of the gradient.) Bacteria (approximately 10^7 viable units per ml) were inoculated into 10 ml DM medium at 30°C . Thymidine and deoxyadenosine were added to give final concentrations of $200 \mu\text{Ci ml}^{-1}$ and $50 \mu\text{g ml}^{-1}$, respectively. After 5 min, half the culture was membrane-filtered and washed with 2 volumes of chilled medium lacking glucose, deoxyadenosine and thymidine (pulse). The other half of the culture was also filtered after 5 min but was washed with 2 volumes of DM medium lacking deoxyadenosine and thymidine at 30°C . This filter membrane was immersed in 5 ml of fresh DM medium lacking only deoxyadenosine and thymidine at 30°C , the cells were detached from it by agitation and the membrane discarded. After 15 min at 30°C the cells were collected again by membrane filtration and washed with 2 volumes of chilled medium lacking glucose, deoxyadenosine and thymidine (pulse chase). The two membranes carrying pulsed and pulse-chased bacteria were resuspended in 1 volume of DM medium lacking glucose, deoxyadenosine and thymidine and the bacteria were detached by agitation. The membranes were discarded and the suspension was centrifuged at $3,000g$ for 10 min. The pellets were each resuspended in 0.6 ml of 0.1 M NaOH containing 10 mM EDTA. λ phage DNA labelled with ^{14}C was added as a 34S sedimentation marker and the mixture was incubated at 37°C for 1 h. After clarification at $3,000g$ for 10 min, the upper 0.4 ml of each supernatant was layered on 13 ml sucrose gradients (5–20%) of 0.9 M NaCl, 0.1 M NaOH and 2 mM EDTA and centrifuged at $38,000 \text{ r.p.m.}$ in an S40/135 rotor for 3 h at 20°C . Thirty fractions were then collected from the top of the gradient and 1 ml of cold 10% trichloroacetic acid added to each. Precipitates were collected on Whatman GF/C filter disks and washed with $5 \times 10 \text{ ml}$ portions of cold 1 M HCl. The filters were dried and radioactivity determined by scintillation counting.

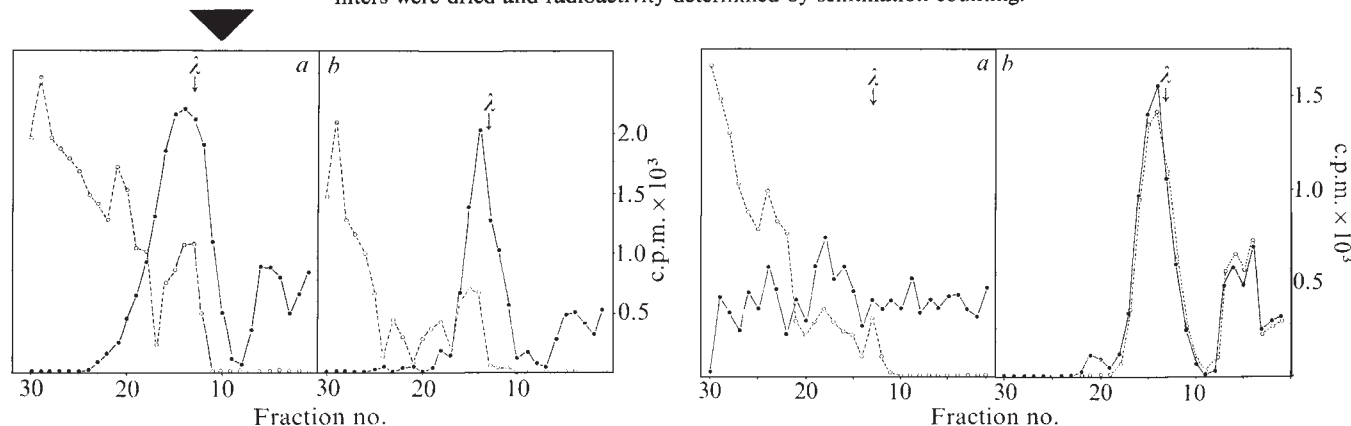


Fig. 3 DNA size distributions. *a*, Distribution of pulse-labelled DNA (●) from strain MH5 obtained by alkaline sucrose density gradient centrifugation; ○, results obtained after a similar pulse followed by a 15 min label-free chase period. *b*: ●, DNA from strain MH5 obtained by alkaline sucrose density gradient centrifugation of a culture treated with NAL ($20 \mu\text{g ml}^{-1}$) for 20 min before pulse labelling; ○, results obtained after a similar pulse followed by a 15-min label-free chase period in the presence of NAL. (Fractions numbered from the top of the gradient.) The experimental details were the same as those described in the legend to Fig. 2.

in the presence and absence of NAL was calculated, the result was surprising. NAL ($20 \mu\text{g ml}^{-1}$) inhibited DNA synthesis by only 14% after 20 min of treatment, whereas the continuous label study had indicated that DNA synthesis was inhibited by about 80% in identical conditions.

Examination of the DNA replication of the *nalA* mutant strain MH5 by pulse labelling showed that this organism accumulated 38S single-stranded DNA fragments even in the absence of the drug (Fig. 3*a*). During the chase period these precursors were converted slowly into DNA of high molecular weight. When the experiment was repeated with strain MH5 treated with NAL ($20 \mu\text{g ml}^{-1}$) for 20 min before a pulse label, the accumulation of DNA precursors and their conversion into DNA of high molecular weight proceeded relatively unimpaired (Fig. 3*b*).

Thus the *nalA* mutation slows the joining of 38S DNA precursors and sensitive bacteria treated with NAL are inhibited at the same step, in that they accumulate 38S DNA fragments which cannot be converted into DNA of higher molecular weight. Thus it seems that the *nalA* gene product has a unique role in the sealing of 38S single-stranded DNA precursors, which seems to be a hitherto unknown vital step in the replication of the bacterial chromosome.

There remains the apparent discrepancy between the continuous and pulse labelling results with strain KL16. When the sequential events of DNA replication are considered, however, the two results can be reconciled as follows.

In bacteria sensitive to NAL, exogenous ^3H -thymidine is incorporated into 10S Okazaki fragments¹¹. These are joined by DNA ligase into longer single-stranded DNA, of molecular weight 38S, which in turn are converted into full-sized daughter molecules. This last step seems to be

governed by the action of the *nalA* gene product. When sensitive bacteria are treated with NAL it is only this step that is inhibited (Fig. 2) so that gaps remain between each 38S single-stranded DNA precursor. Thus NAL does not inhibit the incorporation of ^3H -thymidine into trichloroacetic acid insoluble single-stranded DNA of low or intermediate molecular weight, as measured by pulse labelling. In other words, NAL does not inhibit DNA synthesis. But, the newly synthesised single-stranded DNA precursors, by virtue of the unsealed gaps, are most probably metabolically unstable, as they will be substrates for exonuclease action. Such degradation, which has been described during NAL treatment of *Bacillus subtilis*¹², would return the ^3H -label to the intracellular thymine pool from where it would be recycled into Okazaki fragments. Thus net DNA synthesis would cease because of the transience of the newly synthesised DNA intermediates, and this is the result we obtained with continuous labelling of DNA. Experimental support for these views is given by the finding that competent RNA and protein syntheses are essential prerequisites for both exonuclease action and the bactericidal action of NAL^{8,13}, and thus our results seem not only to provide evidence about the nature of the target site for NAL, but also to indicate how the drug kills bacteria.

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Received November 5, 1975; accepted March 3, 1976.

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π Turn is a conformational pattern in RNA loops and bends

A PATTERN has been found in the structure of transfer RNA by which a polynucleotide chain can make an abrupt change in direction so as to make a sharp cornered loop or a sharp bend.

Nucleotide sequences¹ of transfer RNAs (tRNAs), ribosomal RNAs (rRNAs) and phage RNAs suggest that they can be represented as a series of double-stranded helices with loops separated by short stretches of single-stranded RNA. The single-stranded loops and short stretches have been implicated as the recognition sites for proteins or other nucleic acids.

The three-dimensional structure of yeast tRNA^{Phe} determined by X-ray crystallography provides conformational information about such single-stranded regions. The backbone structure of this tRNA molecule revealed that the loops have sharp corners² rather than being smooth. This feature was verified in detail at 3-Å resolution as well as at higher resolution for the same tRNA in two different crystalline lattices³⁻⁷. Examination of the T ψ C and the anticodon loops, both with seven nucleotides, shows that the way the backbone makes a sharp turn is the same for both loops, suggesting that this is a pattern of forming a sharp cornered loop (Fig. 1). The reversing of

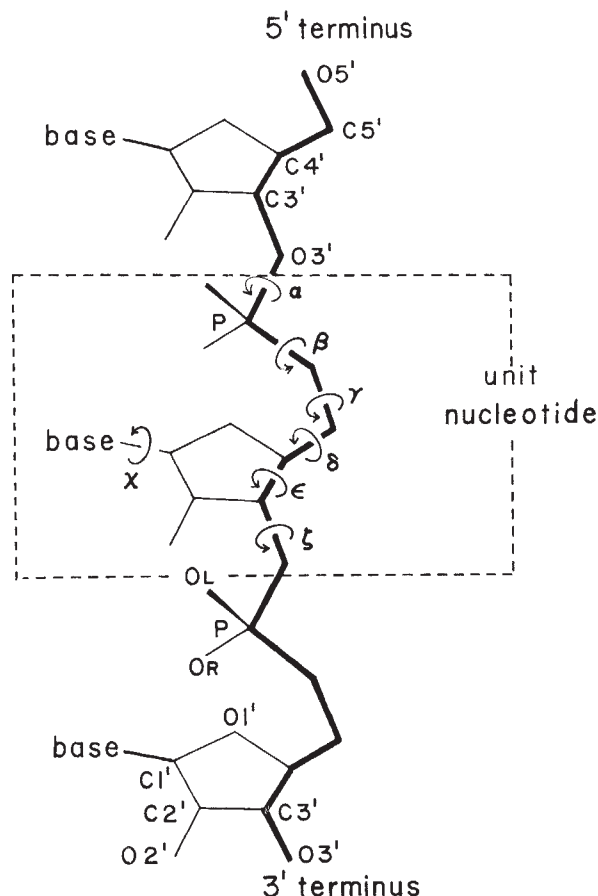


Fig. 2 The nomenclature of torsional bonds used here is a modification from that suggested in ref. 9 to accommodate all the torsional angles in one 'unit nucleotide'.

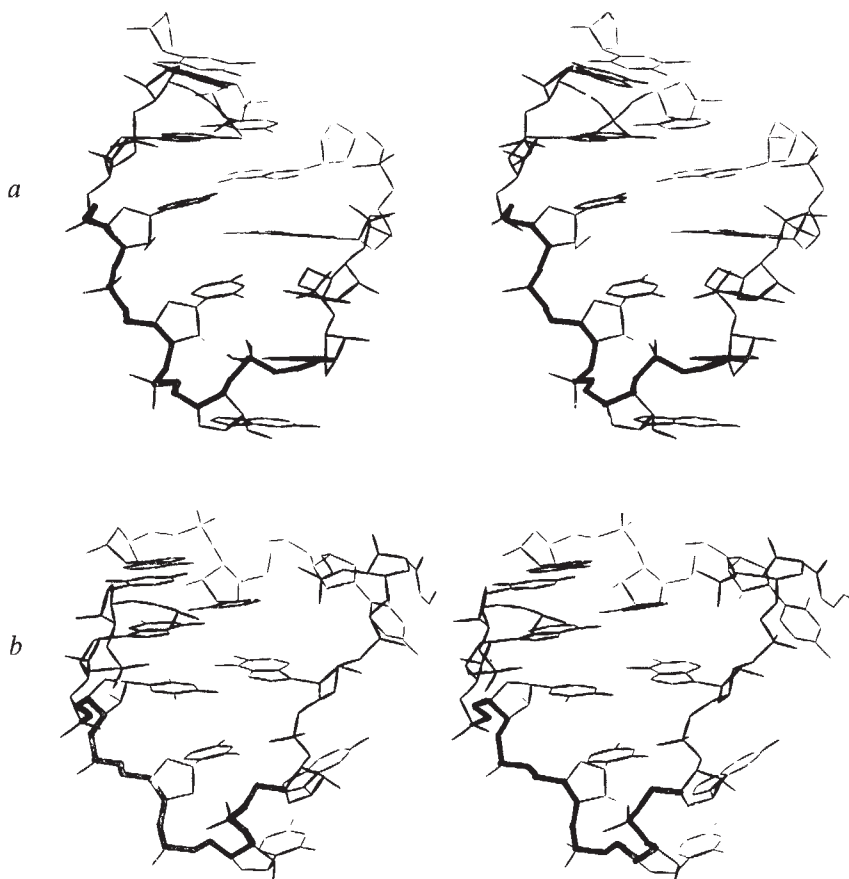


Fig. 1 Stereo view of the anticodon loop (a) and the T ψ C loop (b) of yeast tRNA^{Phe}. At the top of each figure are two base pairs just before the loop. The 5' end is at the upper left and the 3' end at the upper right. The π turn occurs at the phosphodiester link between the bottom nucleoside and that at the 5' side of it. These computer drawings are based on the atomic coordinates of this tRNA structure in the orthorhombic crystal form⁶. The side chain tail of the Y base has been omitted from (a) for clarity, and the backbone of the 5' half of the loops are darkened.

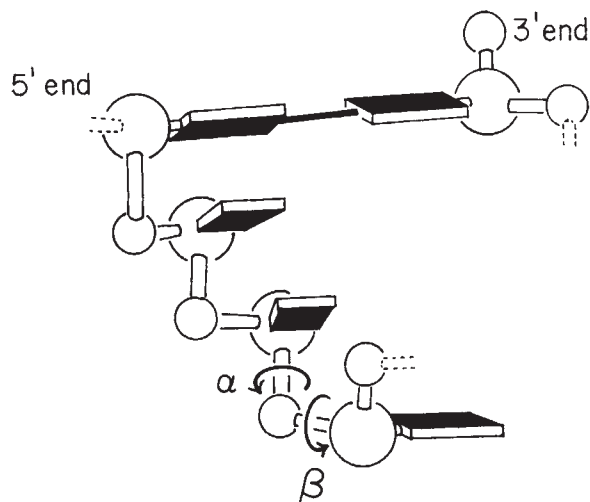


Fig. 3 Schematic drawing of the common portion of Fig. 1a and b. The circular arrows indicate two bonds involved in π turn to reverse the direction of the backbone from a helical conformation.

the direction of the backbone chain from the helical conformation occurs, in both cases, within just one phosphodiester link by rotating primarily around O3'-P and P-O5' (torsion angles α and β , see Fig. 2) of the third nucleotide from the 5' end of the loop. These two phosphodiester bonds were initially pointed out to be the two least constrained backbone torsion angles⁸. This is shown schematically in Fig. 3. Besides these two examples there are several more sharp turns formed by similar rotations around the α and β in the DHU loop, the variable loop, between the helices and in the T ψ C loop.

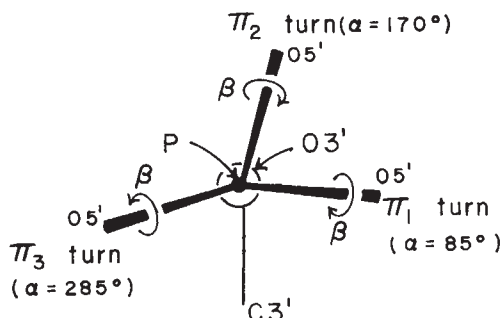


Fig. 4 Three families of π turns, as distinguished by their α angles, are viewed along the P-O3' bond. The sharpness of the turn is dictated by how much the β angle deviates from a helical conformation.

For convenience, we define the π turn to describe a set of sharp turns in a polynucleotide chain, all due to the rotation around two adjacent phosphodiester bonds, causing changes in the direction of the backbone chain. There are three possible families of π turns, depending on the orientation of the P-O5' bond with respect to the C3'-O3' bond, that is the torsion angle α , as shown in Fig. 4: called π_1 , π_2 and π_3 turns with α values near 85°, 170° and 285° respectively. The three representative α angles chosen here are based on the observed values in crystal structures of several dinucleoside phosphates⁹. The most common turn found in yeast tRNA^{Phe} is the π_3 turn. This is observed in the phosphodiester links between residues 9 and 10, 17 and 18, 33 and 34, 46 and 47 and 55 and 56. The sharpness of the turn depends on how much the β angle deviates from a helical conformation (near 290°) (ref. 9). The other two classes of π turns are also found in this tRNA structure; for example, π_1 turn between 47 and 48; π_2 turn between 15 and 16. There are several more π turns in the tRNA structure which are uncertain, at the current stage of refinement, to assign a specific class.

The π_1 turn has been observed in the crystal structures of uridylyl 3',5'-adenosine phosphate, UpA^{10,11}, ApApA¹² and ApU-9-aminoacridine¹³ where two oppositely oriented riboses are connected by one phosphodiester linkage.

Thus, it seems that the π turn provides a simple, general pattern by which a polynucleotide chain makes a sharp turn, either to form a loop or to change the direction of the chain flow. Such a sharp cornered loop or turn produces a small compact protrusion, which could fit into a depression or groove on the surface of a protein with which it interacts.

This work was supported by the US National Science Foundation and the National Institutes of Health. J.L.S. is a fellow of the Arthritis Foundation.

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Received November 13, 1975; accepted February 17, 1976.

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Systematic nomenclature for the RNA polymerase genes of prokaryotes

THE DNA-dependent RNA polymerase of *Escherichia coli* (EC 2.7.7.6) consists of at least four distinct polypeptide subunits; α , β , β' and σ (ref. 1). Other subunits have been reported (for example, ω (ref. 1) and σ' (ref. 2)), but their importance in transcription remains uncertain. Many prokaryotes are known to contain RNA polymerases of closely similar subunit composition, including a wide range of bacteria³ and a blue-green alga⁴. Until recently, only the structural gene for β had been identified in *E. coli*⁵. It is located⁶⁻¹⁰ at 88.5 minutes on the recalibrated genetic map¹¹, and is characterised principally by mutations conferring resistance to specific drugs. The β -gene has thus acquired several synonyms derived from the mutant phenotypes, including *rif* (for resistance to rifampicin)⁸, *stv* (for streptovaricin)⁹, and *stl* (for streptolydigin)^{10,12}. More recently the structural gene for the β' subunit has been characterised in *E. coli*; it is adjacent to the β gene¹³, and in the same operon¹⁴⁻¹⁶. Finally, the gene coding for α has been located at 72 minutes^{11,17-19}. We now propose a systematic nomenclature for the RNA polymerase genes of *E. coli*, which could equally be applied to other prokaryotic organisms. We have consulted other workers in the field, whose comments have been assimilated into our proposal. The scheme, which is in line with the recommendations of Demerec *et al.*²⁰, is as follows.

(1) The symbol *rpo* should be applied to the structural genes coding for RNA polymerase subunits, and to their regulatory genes and sites. (The symbol *rna*, proposed in 1968 (ref. 7), has not come into general use²¹.)

(2) The genes for the α , β and β' subunits should be called *rpoA*, *rpoB* and *rpoC*, respectively. We suggest that

Table 1 Revised names of some RNA polymerase mutations

New name	Former name	References
	<i>Escherichia coli</i> K12	
<i>rpoA109</i>	<i>gro-109</i>	18, 19, 21
<i>rpoB1</i>	<i>uvr1</i>	16
<i>rpoB2</i>	A2	16
<i>rpoB3</i>	<i>rif^a</i>	22
<i>rpoB4</i>	<i>rif-r4</i>	23
<i>rpoB5</i>	<i>rif-r-5</i>	24
<i>rpoB6</i>	<i>rif-6</i> or <i>rif^a_{RCB6}</i>	25
<i>rpoB7</i>	7	16
<i>rpoB8</i>	K _M 7	26
<i>rpoB9</i>	XR9	16
<i>rpoB10</i>	<i>rif-r_d</i>	27
<i>rpoB11</i>	<i>rif-r-1</i>	24
<i>rpoB12</i>	<i>rif^a_{amD12}</i>	14, 16, 28
<i>rpoB15</i>	<i>rif^a_{amB15}</i>	28, 29
<i>rpoB17</i>	XR17	16
<i>rpoB18</i>	<i>stl-r-18</i>	30, 31
<i>rpoB19</i>	<i>rif-r-18</i>	30
<i>rpoB20</i>	BT20	16
<i>rpoB21</i>	(<i>stl^R</i>)	10
<i>rpoB22</i>	ts22	30, 31
<i>rpoB25</i>	<i>rif-rB5</i>	32
<i>rpoB35</i>	ST35	16
<i>rpoB38</i>	<i>rif^a_{amIII/8}</i>	14, 16, 28
<i>rpoB44</i>	ST44	16
<i>rpoB49</i>	ST49	16
<i>rpoB64</i>	<i>rif-r64</i> (SD)	33
<i>rpoB65</i>	ST65	16
<i>rpoB70</i>	<i>rif-rJ7</i>	28, 32
<i>rpoB71</i>	ST71	16
<i>rpoB74</i>	ST74	16
<i>rpoB76</i>	<i>rif-r-76</i>	34
<i>rpoB80</i>	ST80	16
<i>rpoB90</i>	ST90	16
<i>rpoB91</i>	ST91	16
<i>rpoB93</i>	J93	16
<i>rpoB96</i>	ST96	16
<i>rpoB101</i>	ST101	16
<i>rpoB110</i>	ST110	16
<i>rpoB115</i>	<i>rif^aB115</i> amber	35
<i>rpoB122</i>	R122	16
<i>rpoB123</i>	R123	16
<i>rpoB124</i>	R124	16
<i>rpoB130</i>	R130	16
<i>rpoB143</i>	ts143	30, 31
<i>rpoB165</i>	ts65	30, 31
<i>rpoB171</i>	ts71	30, 31
<i>rpoB184</i>	D184	16
<i>rpoB195</i>	<i>stv195</i>	36
<i>rpoB201</i>	<i>stl1</i>	12
<i>rpoB203</i>	XH203	16
<i>rpoB245</i>	XH245	16
<i>rpoB274</i>	XH274	16
<i>rpoB347</i>	XH347	16
<i>rpoB501</i>	<i>rif501</i>	37
<i>rpoB507</i>	<i>rif507</i>	38
<i>rpoB514</i>	R514	16
Δ (<i>argBH-rpoB</i>) 18	Δ 18	14, 16
<i>rpoC1</i>	tsX	39, 13
<i>rpoC2</i>	B2	16
<i>rpoC3</i>	cs1	23
<i>rpoC4</i>	4, ts-4	16, 40
<i>rpoC17</i>	XH17	16
<i>rpoC25</i>	XH25	16
<i>rpoC38</i>	B38	16
<i>rpoC47</i>	B47	16
<i>rpoC55</i>	ST55	16
<i>rpoC56</i>	XH56	16
<i>rpoC87</i>	ST87	16
<i>rpoC110</i>	XH110	16
<i>rpoC120</i>	R120	16
<i>rpoC202</i>	XH202	16
<i>rpoC212</i>	XH212	16
<i>rpoC233</i>	XH233	16
<i>rpoC254</i>	XH254	16
<i>rpoC284</i>	XH284	16
<i>rpoC385</i>	XH385	16
	<i>Salmonella typhimurium</i> LT2	
<i>rpoB4</i>	<i>rif4</i>	*
<i>rpoC32</i>	ts32	*
	<i>Bacillus subtilis</i> NCTC 3610	
<i>rpoB3</i>	<i>rfm3Y</i>	41

*Personal communication from A. Wright.

the gene for σ be *rpoD*, and that any further structural genes should continue the alphabetical sequence.

(3) The promoter for the *rpoB,C* operon¹⁴ should be designated *rpoP*. We suggest that further regulatory genes and sites, controlling *rpo* structural genes, should continue the alphabet from *rpoO*.

(4) All *rpo* mutations should be identified by the gene letter, followed by a number. We strongly support the principle²⁰ that allele designations should not attempt to convey phenotypic information.

Table 1 lists some well-characterised mutations established in the literature, together with their agreed new names. We will gladly coordinate the future naming of mutations.

Table 1 lists some well-characterised mutations established *E.coli*¹¹.

We thank many colleagues for support and help in the shaping of this nomenclature, particularly the following: S. J. Austin, B. J. Bachmann, D. Boyd, R. R. Burgess, R. Calendar, I. V. Claeys, R. Doi, H. Echols, G. R. Hartmann, A. Heil, A. Ishihama, R. B. Khesin, J. B. Kirschbaum, J.-P. Lecocq, R. Losick, B. Low, H. Matzura, D. McConnell, J. H. Miller, B. Molholt, M. Nomura, M. Oeschger, P. Palm, L. G. Silvestri, A. L. Sonenshein, W. Szybalski, A. L. Taylor, I. Tittawella, A. Travers, A. Wright, T. Yura and W. Zillig. We thank the MRC for project and programme grant support.

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Enthalpy-entropy compensation of oxamate binding by homologous lactate dehydrogenases

STUDIES^{1,2} of the amino acid sequence and crystal structure of M₄ lactate dehydrogenase (EC 1.1.1.27) indicate that all binding interactions are of the weak, non-covalent type (proceeding with only minor free energy changes). Because critical regions of the substrate and coenzyme-binding site seem highly conserved³, and because non-covalent interactions are highly, if differentially, sensitive to the physical environment⁴, we wondered how organisms living in widely different temperatures and pressures stabilise enzyme-ligand interactions. We have therefore compared pyruvate binding site functions of lactate dehydrogenases obtained from organisms living in different temperatures and pressures. We used a placental mammal, the ox, with a cell temperature of 37 °C; a marsupial, the possum (*Trichosurus vulpecula*), with an average cell temperature of 36 °C (ref. 5), two monotremes, the platypus (*Ornithorhynchus anatinus*), with a cell temperature of about 31 °C (ref. 6), and the echidna (*Tachyglossus aculeatus*), with a body temperature of 23–32 °C (ref. 7); the goanna (*Varanus gouldi*), an Australian lizard with a cell temperature ranging from about 20 up to nearly 40 °C (ref. 8); the Pacific coast dogfish (*Squalus acanthias*), with an average body temperature of about 15 °C; an intertidal sculpin (*Oligocottus maculosus*), living in varying temperatures between about 5 and 15 °C, and an abyssal teleost fish, *Antimora rostrata*, with a highly constant body temperature of 2–3 °C. All these, except the dogfish and *Antimora*, can be viewed as species adapted to 1 atm; by contrast the abyssal species must be able to tolerate pressures of up to several hundred atmospheres, while the dogfish may encounter pressures from 1 to about 100 atm (N. J. Wilimovsky, personal communication). We found that adjustments in enthalpic and entropic contributions to lactate dehydrogenase binding allowed each homologue to bind ligand with similar avidity at its respective "normal" temperature and pressure.

M₄ lactate dehydrogenase was isolated from skeletal muscle of each species, and in all but the dogfish and sculpin, was purified to homogeneity (thrice crystallised) by standard purification procedures as before⁹. Dogfish and sculpin enzymes were purified by affinity chromatography using oxamate covalently linked to Sepharose B as described elsewhere¹⁰. Enzyme activity was assayed by following the change in A₃₄₀ due to NADH oxidation in a Unicam SP 1800 recording spectrophotometer. Cuvette temperatures were kept constant

(±0.1 °C) with a Lauda constant temperature bath and circulator. To study the effects of pressure, we used a high pressure cell of 1-cm light path, with sapphire windows, installed into a Unicam SP 1800 spectrophotometer, as previously described¹¹.

An indication of how the substrate-binding site is adjusted with respect to the environment can be obtained from studies of

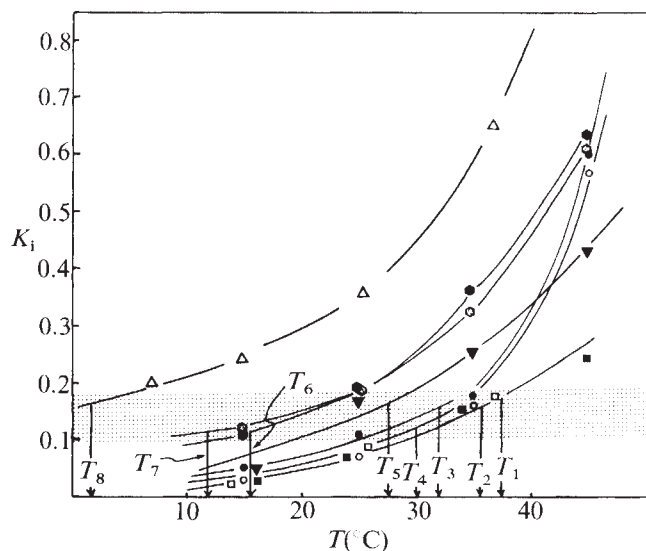


Fig. 1 A plot of the K_i for oxamate against temperature in °C for M₄ lactate dehydrogenases from eight vertebrate species. Arrows from each curve refer to the approximate body temperature in each case. T_1 refers to the ox; T_2 , the possum; T_3 , the platypus; T_4 , the goanna; T_5 , the echidna; T_6 , the dogfish; T_7 , the sculpin; T_8 , *Antimora*. The K_i for oxamate was determined at pH 7.5, 100 mM Tris-HCl buffer, 25 °C, at minimally two concentrations of pyruvate, between 0.25 mM and 1 mM, and at 0.1 mM NADH. Total volume was 1 ml. Pressure was constant at 1 atm. K_i values (mM) are accurate to within ±10%. Each K_i value represents an average of three determinations from Dixon plots of 1/velocity against oxamate concentration.

oxamate inhibition of the enzyme. Oxamate, isoelectronic and isosteric with pyruvate, is considered a perfect structural analogue of the substrate². During oxamate or pyruvate binding, a negative charge must be neutralised, and the overall process should be favoured by low temperature and low pressure⁴. If that were simply the case, then the lower the temperature, the higher the enzyme affinity for oxamate, and the enzyme from a low temperature organism (such as *Antimora*) would be expected to have a much higher affinity at its biological temperature than would a homologue in a high temperature organism. This situation was not observed. Although the overall effect of temperature on the K_i for oxamate is qualitatively similar, the position of each curve on the temperature axis

Table 1 Thermodynamic parameters for association of oxamate and binary lactate dehydrogenase-NADH complexes for M₄ forms of the enzyme from species adapted to different temperatures and pressures

Enzyme source	Average biological temperature (°C)	ΔG^0 (kcalorie mol ⁻¹)	ΔH^0 (kcalorie mol ⁻¹)	ΔS^0 (calorie mol ⁻¹ K ⁻¹)	ΔV^0 (ml mol ⁻¹)
Ox	37	-5.7	-15.4	-32.3	36
Possum	36	-5.7	-15.2	-31.9	Unknown
Platypus	31	-5.4	-14.5	-30.5	Unknown
Goanna	20–40	-5.6	-14.5	-29.9	Unknown
Echidna	23–32	-5.2	-14.0	-29.5	Unknown
Dogfish	15	-5.1	-11.0	-19.8	5.4
Sculpin	5–15	-5.1	-8.2	-11.1	10.0
<i>Antimora</i>	2	-4.7	-6.1	-4.9	0

K_i values from which these data are calculated are reproducible to within ±10%. Values for ΔG^0 , ΔS^0 and ΔV are calculated for data obtained at 298 K.

roughly correlates with the temperature in which each enzyme homologue normally functions (Fig. 1). In each case the affinity of the binary complex for oxamate is similar at each biological temperature (although Fig. 1 shows that at any given temperature enzymes adapted to high temperatures bind oxamate more tightly than do their homologues in organisms adapted to the cold).

Similar observations have been made for the apparent Michaelis constant for pyruvate¹². These and our own studies imply some selective advantage if, in each species, these enzymes have a particular range of substrate affinity. Atkinson has argued persuasively that an enzyme's affinity for its substrates is designed by natural selection for optimal function within the *in vivo* concentration range through which the substrates fluctuate¹³; since pyruvate concentrations are likely to be

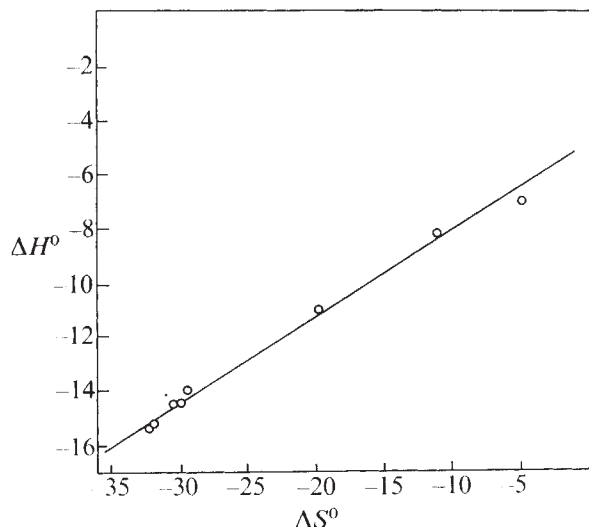


Fig. 2 A plot of ΔH° against ΔS° associated with oxamate-binding by the binary NADH-enzyme complex for lactate dehydrogenases isolated from vertebrate species adapted to different physical environments. The enthalpy change is in calorie mol^{-1} while the entropy change is in calorie $\text{mol}^{-1} \text{K}^{-1}$. The data for this plot are calculated from K_i values that are reproducible to within $\pm 10\%$.

similar in muscles of different vertebrates, similar binding ability at each respective biological temperature should perhaps be expected. Be that as it may, the question remains as to how such fine thermal adaptation of lactate dehydrogenase-binding function is achieved, particularly with a ligand as apparently simple as oxamate.

One possible mechanism would involve structural adjustments at the substrate binding site *per se* so as to 'optimise' binding at different biological temperatures. Although this may be the simplest hypothesis to fit the data, current evidence indicates that critical regions of the enzyme-binding sites are highly conserved¹⁻³. Therefore, we tentatively reject the hypothesis.

A second basis for this rejection perhaps is to be found in a careful analysis of the thermodynamic parameters for association of oxamate and the binary complex. Table 1 shows that the lower the biological temperature of normal function, the lower the enthalpy and entropy changes occurring during oxamate binding. Although the differences between some of the enzyme homologues are small, at the extremes of the spectrum obtained the differences are impressive: the values for ΔH° for oxamate binding by the possum or ox homologues are nearly 1.5 times the ΔH° for the dogfish enzyme and are 2.5 times the ΔH° for the *Antimora* M_4 enzyme. The quantitative relationship between ΔH° and ΔS° for oxamate binding by the different lactate dehydrogenases (Fig. 2) seems to be an example of "enthalpy-entropy compensation". The slope of the plot in Fig. 2 corresponds

to a "compensation temperature" of about 325 K, and is similar to that obtained for ADP binding by glutamate dehydrogenase¹⁴ and for activation parameters for vertebrate lactate dehydrogenases^{15,16}. The compensation temperature range, 250–325 K, is observed so frequently that some workers believe the phenomenon to be a consequence of the properties of water as a solvent^{17,18}. In general, the more water that must be removed from the substrate or from the enzyme, the higher the enthalpy and entropy changes during formation of an enzyme-substrate complex. That some such effects contribute to the thermodynamics of coenzyme and substrate binding by lactate dehydrogenases is supported by several observations. First, in the ternary complex, the position of a collapsed loop excludes bulk water from the active site, while in the apoenzyme it extends well into the solvent¹. In this sense at least water must be removed from the enzyme during stabilisation of the ternary complex. Second, water must also be removed from the coenzyme during binding because both negative charges on the pyrophosphate are solvated in solution, but only one is solvated in the ternary complex^{1,2}. Third, the degree of surface hydration of the apoenzyme compared with the ternary complex almost certainly differs significantly, although structural studies of lactate dehydrogenases have not quantified this characteristic. Fourth, enzyme-water interactions seem to vary between species, with homologues from cold-adapted species tending to have notably oily surfaces, a characteristic observed for both pyruvate kinases and glyceraldehyde-3-phosphate dehydrogenase (G. N. Somero, personal communication). Thus there seems ample possibility to adjust the thermal dependence of enzyme-ligand binding by adjusting the energetic contributions of solvation effects anywhere in the enzyme structure.

If that were true in our case, volume changes associated with oxamate-binding by lactate dehydrogenase homologues should also vary in a regular manner, the larger the ΔH° and ΔS° , the larger the expected volume change (ΔV) (ref. 17). At least in a qualitative sense, this prediction is realised in preliminary studies of total volume changes during oxamate binding. Thus the expected volume increase, associated with charge neutralisation⁴, appears with M_4 homologues from high cell temperature organisms¹⁹. In contrast, oxamate binding by the piscine binary complexes proceeds with a greatly reduced volume change (Table 1); with *Antimora* enzyme the ΔV is so close to zero it cannot be measured accurately by the techniques used. These studies are in progress and will be published in detail elsewhere. In these cases the simplest interpretation is that the positive ΔV occurring on charge neutralisation of oxamate is at least partially compensated (or accommodated) by a negative volume change of similar magnitude, but mediated by changes in the degree to which water can orient around water-density-modifying groups such as carboxylates, peptide linkages, or non-polar amino acid residues. In consequence, the total ΔV is lower than in the ox homologue. As with activation parameters²⁰, nothing need be assumed about where in the enzyme structure these events occur, although they probably occur somewhere removed from critical interacting regions of the substrate-binding site *per se*.

This work was supported by a grant (P.W.H.) from the National Research Council of Canada. We thank Dr Paul Dreizen for the *Antimora*.

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Possible Gibbs–Donnan mechanism for cation uptake

MANY freshwater animals take up metal ions from solution in the surrounding water, often by active transport against a concentration gradient. The uptake of ions would be helped, and their loss opposed, by any means which increases the electrochemical potential of the ions in question in the external medium. One such means would be for the animal to secrete into the water a cationic substance to which the skin is not permeable; this would set up a Gibbs–Donnan effect (whereby an unequal distribution of diffusible ions is established at equilibrium on the two sides of a membrane if one side contains a non-diffusible ion) on the ions which are to be absorbed. The presence of such a mechanism in nature would be evident from the accumulation in the growth medium of cationic substances whose effect on the uptake of metals could be demonstrated experimentally.

For instance, the pulmonate snail *Biomphalaria glabrata* was shown by Thomas *et al.*^{1,2} to secrete a substance which might act this way. The growth of the snails in these experiments was on the whole limited by the amount of calcium which could be absorbed, but the snails' secretion increased their growth rate and lowered the minimum concentration from which net uptake of calcium could be achieved. When the substance was allowed to accumulate in a limited volume of water (< 200 ml) for 3 d, the calcium concentration was reduced by the snails' uptake to $\sim 2.5 \times 10^{-5}$ M. But when the secretion was prevented from accumulating by continuous replacement of the medium, net uptake was not possible from solutions $\lesssim 2.5 \times 10^{-4}$ M. Addition of the partially purified secretion of other snails to the continuously replenished medium lowered the limiting calcium concentration from which net uptake was possible.

This model implies that the secretion of *B. glabrata* could increase the electrochemical potential of calcium tenfold. It is possible to calculate the effective concentration of non-permeant cations required to produce this effect, assuming that no other cations are present in significant amount. We can treat the system as though Ca^{2+} were partitioned at equilibrium between two compartments separated by a membrane permeable only to Ca^{2+} and to a common anion such as HCO_3^- . The molar calcium concentration in the compartment from which the non-permeant cation is absent (representing the interior of the snail) would then be $m'_{\text{Ca}} = 2.5 \times 10^{-4}$ and in the compartment where the non-permeant cation is present (representing the exterior medium) the calcium concentration is $m_{\text{Ca}} = 2.5 \times 10^{-5}$. If the substance in question has molecular charge z , the molar concentration, m_p , required will be given by the Gibbs–Donnan equation

$$\gamma_{\pm}^3 m_{\text{Ca}} (2m_{\text{Ca}} + zm_p)^2 = 4\gamma_{\pm}'^3 m_{\text{Ca}}'^3$$

where $\gamma_{\pm}$, $\gamma_{\pm}'$ are the activity coefficients of the calcium salt in the compartments with and without the non-permeant cation, respectively. Substituting the calcium concentrations given above, and assuming the activity coefficients equal, we obtain the value $zm_p = 1.6 \times 10^{-3}$, which seems reasonable.

The Gibbs–Donnan effect will operate on all permeant cations, to an extent depending on their relative concentrations and their valence. (There is more effect on multivalent than on univalent ions.) The presence of an excessive concentration of another permeant ion such as Na^+ would reduce the enhancement of the electrochemical potential of Ca^{2+} . Other things being equal, the uptake of permeant cations would be favoured in approximate proportion to their external concentrations. Other non-permeant ions would enhance or reduce the Gibbs–Donnan effect depending on their concentrations inside and outside the animal and on their net molecular charges.

Apart from the identification of cationic macromolecular substances in the secretions of freshwater animals, this model could be confirmed by reproducing the Gibbs–Donnan effect with cationic polymers such as diethylaminoethyl dextran or polylysine. The presence of these in the water at quite modest concentrations ought to assist the uptake of calcium and stimulate the growth of animals as does the substance studied by Thomas.

It is not to be expected that such a mechanism would be useful to all freshwater animals, but only to those living in limited volumes of still water in which the hypothetical substances could accumulate. Because the mechanism would act equally well to retard loss as to promote uptake of ions, it would be of greater value to animals vulnerable to such loss; for example, it would be more likely to occur among gastropods, which are liable to dissolution of the shell, than among crustaceans, whose calcareous structures are protected by an impermeable cuticle.

I am indebted to Dr A. G. Ogston for his suggestions and criticisms.

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Received January 26; accepted February 26, 1976.

¹ Thomas, J. D., Benjamin, M., Lough, A., and Aram, R. H., *J. Anim. Ecol.*, 43, 839–860 (1974).

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matters arising

Evidence for two sodium sites on the external aspect of Na-K pump in human erythrocytes

THE Na-K pump, which actively transports Na outward and K inward, is inhibited by external Na in human erythrocytes. This inhibition has been interpreted as being either "dead end" at one of the two putative external K sites¹, or as allosteric, occurring at a third external site on the pump². Both of these models assume that occupation of the pump by a single external sodium ion is

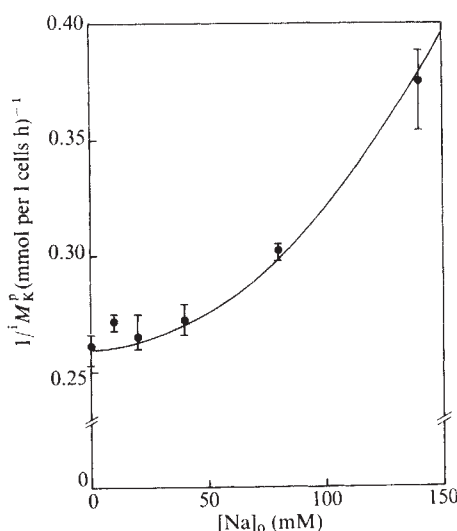


Fig. 1 Inhibition of ouabain-sensitive K influx by external Na in human red cells. Cellular cation concentrations were altered by reversibly increasing cation permeability using nystatin⁴, so that $[Na]_i = 88$ mmol per 1 cells and $[K]_i = 8$ mmol per 1. Unidirectional K influxes were measured using ^{42}K as before⁵ in a solution containing (mM): KCl, 2; choline Cl+NaCl, 148; glucose, 5; Tris-HCl, 10 (pH 7.5). The cells were exposed to ^{42}K for 30 min. The maximum difference in $[K]_i$ s in aliquots of cells at the various $[Na]_o$ s, measured after incubation in ^{42}K , was 0.9 mmol l⁻¹. The resultant differences in ^{42}K due to stimulation by $[K]_i$ would be of the order of 5% (ref. 6). Ouabain-sensitive fluxes were determined after preincubation of cells in 10^{-9} M ouabain for 10 min at 37°C in K-free media. Results are plotted as the reciprocal of the pump influx, $1/M_K^p$, against external Na concentration, $[Na]_o$. The curve was drawn from the equation $1/M_K^p = 0.26 + (6.0 \times 10^{-6}) [Na]_o^2$. The points represent means of triplicate determinations. The maximum range of values about a mean was 3.9% of the mean, and the average range was 2.4%. Similar results were obtained in another comparable experiment.

sufficient to inhibit the pump. In the "dead end" model, occupation of one of the K pump sites by Na inhibits pump turnover¹; in the allosteric model, occupation of the external sodium site decreases the affinity of the K pump sites for K, but does not inhibit K translocation once the K sites have been occupied.

The experiments which led to these models have been done at relatively low external K concentrations, $[K]_o$, well below 1.5 mM, the $[K]_o$ at half-maximal pump flux in high Na solutions³. When inhibition by Na of ouabain-sensitive K influx, M_K^p , was determined in solutions containing 2 mM $[K]_o$, results such as those in Fig. 1 were obtained. The results, presented as a Dixon plot⁷ ($1/M_K^p$ against $[Na]_o$), fit a parabola, suggesting that two external sites must be occupied for Na to inhibit ouabain-sensitive K influx at the $[K]_o$ used. These results contrast with those obtained by Cavieres and Ellory² at lower $[K]_o$. Dixon plots of their data gave straight lines for K concentrations between 0.1 and 0.35 mM, and hyperbolae for concentrations less than 0.1 mM. The difference in the results at 2 mM $[K]_o$ compared with lower $[K]_o$ s might be considered in terms of the relative affinities of the two sodium sites depending on whether one K ion or two occupy the external aspect of the pump. The form XKX may have such a high affinity for Na at the first Na site that the effect of occupation of this site is not seen at the lower $[K]_o$ s. This would be analogous to measuring K activation of the pump in Na-free solutions where the curve appears hyperbolic unless extremely low $[K]_o$ s are used, where a slight sigmoidicity may become apparent. In this scheme the form KXX would have a lower affinity for Na than XK and the occupation of both Na sites would be demonstrable kinetically at a higher $[K]_o$.

Another useful way of examining extracellular Na sites on the pump is to look at the effects of $[Na]_o$ on the rate of ouabain binding. External Na increases the rate of ouabain binding to the pump, whereas external K inhibits it. The effect of Na has been attributed both to a direct action, independent of K⁸, and to an action limited to restricting access of K⁺ ions from the sites at which they inhibit ouabain binding¹. In the experiment shown in Fig. 2, we altered the rate of ouabain binding (shown as the rate constant of binding), but not the K pump rate, by increasing $[Na]_o$ in a solution in which $[K]_o$ was sufficient to

saturate the pump at all Na concentrations used. Thus, increasing $[Na]_o$ caused no inhibition of M_K^p , but the rate of ouabain binding increased fourfold. This shows that Na may occupy at least one external site without inhibiting the pump, and supports a configuration of the external aspect of the pump, KXK^{Na} , suggested by Cavieres and Ellory². Figure 1, however, demonstrates that at least two external sites must be occupied by Na to obtain inhibition at 2 mM $[K]_o$. In low $[K]_o$ and saturating $[Na]_o$, one would expect

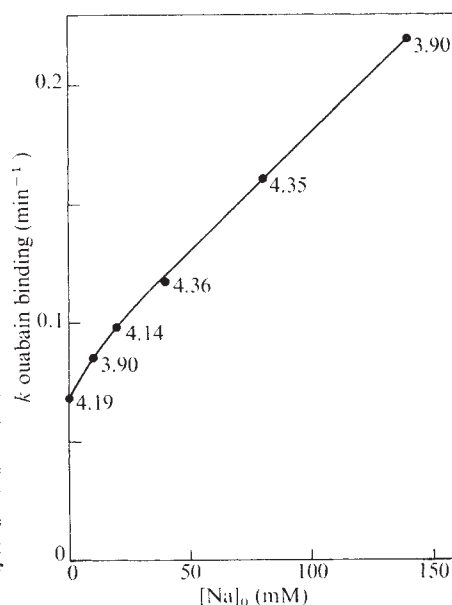


Fig. 2 Promotion of the rate of ouabain binding by external sodium. Cellular cation concentrations were altered using nystatin as described in Fig. 1 so that $[Na]_i = 53$ mmol per 1 cells and $[K]_i = 43$ mmol per 1. The rate of ouabain binding was determined by measuring the onset of inhibition of the pump after preincubation for various times in solutions containing 10^{-8} M ouabain, 11 mM KCl, 5 mM glucose, 10 mM Tris-HCl (pH 7.5) and NaCl as indicated, with NaCl+choline Cl = 140 mM. Ouabain binding was stopped by addition of ice-cold isotonic choline Cl. After washing by centrifugation, unidirectional influx of K was measured⁵ in a medium containing 11 mM KCl, 140 mM choline Cl and Tris-HCl and glucose as before. Thus for each Na concentration a time course of ouabain binding was measured, and the rate constant, k (min⁻¹), of binding was determined from the slope of the curve for the time course. The numbers on the figure are ouabain-sensitive K influxes (in mmol per 1 cells × h) measured in 11 mM $[K]_o$ and Na concentrations indicated but in the absence of ouabain. Fluxes were determined in triplicate and ouabain binding rates in quadruplicate.

the two sites to be filled by Na. Given the "limiting non-zero ouabain-sensitive K influx" described by Cavieres and Ellory in these conditions², the minimum requirement of a model for the pump is that two sites can be occupied by Na and one by K simultaneously. Two possibilities are that (1) the external pump form ${}^{\text{Na}}\text{X}_{\text{K}}{}^{\text{Na}}$ is capable of active K translocation, or (2) additional K or Na sites (for example, the form ${}^{\text{Na}}\text{X}_{\text{K}}{}^{\text{Na}}$) are present on the external aspect of the pump.

This work was supported by a grant from the National Institutes of Health.

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CAVIERES AND ELLORY REPLY—The finding of an upwards deflection of the $1/v$ against $[\text{Na}]_0$ curve would certainly be very interesting. Unfortunately the deviation from a straight line in the data presented¹ is effectively based on only one point ($[\text{Na}]_0 = 140 \text{ mM}$), where the flux is 13.6% lower than it would be for a straight line fit. From the legend to Fig. 1 it is apparent that cell K ($\sim 8 \text{ mM}$) can be $\sim 10\%$ lower in the cells incubated in 140 mM Na medium, thus reducing the K influx by $\sim 5\%$ (ref. 6). If the further contributions of experimental error (up to 3.9%) and the scatter (for example, for $[\text{Na}]_0 = 10 \text{ mM}$) are considered, it becomes difficult to defend the departure from the straight line fitted through the remaining data. In fact, from inspection of our earlier experiments carried out at a $[\text{K}]_0$ of 1 mM, we could find no evidence for deviation from a linear relationship. A final possibility (although unlikely), is that the difference in internal K between our experiments ($\text{K} \sim 50 \text{ mM}$) and theirs ($\text{K} \sim 8 \text{ mM}$) may have a role.

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¹ Hobbs, A. S., and Dunham, P. B., *Nature*, **260**, 651 (1976).

Haze in the stratosphere from the Fuego eruption

SCORER¹ reported seeing an extended haze layer during Concorde flights at heights up to 17 km (56,000 feet) on September 1,

1975 from London to Gander and back. He assumed that this was the result of large scale ascent of normal tropospheric dust. On the premise that this was indicative of the normal interchange mechanisms across the tropopause, he then concluded that the theories which have led to the recent 'ozone depletion scares' are based on 'nonsense' suppositions regarding the nature of the vertical transport in the stratosphere.

A much more likely explanation of the haze layer observed by Scorer is that this was aerosol remaining from the material injected into the lower stratosphere by the violent eruptions in mid-October 1974 of Volcan de Fuego in Guatemala. A number of different investigators reported the appearance of a haze layer (or layers) between ~ 16 and 20 km during the following months². Further observations during the past year, with lidar³, twilight measurements⁴, dust sonde⁵, and search-light measurements⁶ indicate the continued presence of this dust layer over Hawaii, Puerto Rico, and northern mid-latitudes. It is probably still present over the Arctic, but it is not known whether any dust reached the Southern Hemisphere. According to the lidar data the layer has diminished slowly since early spring 1975 with a residence time of ~ 6 months—about half that of large volcanic events. Measurements at several locations show, however, that purple twilight glows are now (after a minimum in April) nearly as strong as those of winter 1974–75. The haze phenomenon described by Scorer has often been studied in colour photographs obtained by time lapse cameras flown on balloons, mostly over New Mexico⁷. In the flights from December 1974 to the most recent in October 1975, strong haziness near the solar azimuth extends to $\sim 20 \text{ km}$. If the Sun is not high up, a rather sharply bottomed haze band appears at a balloon altitude of $\sim 16 \text{ km}$. When the balloon is in the main layer, the distant horizon disappears, and thin layers may be noticed briefly. When the balloon is leaving the layer the distant haze top

becomes sharp and edged by deep blue sky. Optical measurements showed very little aerosol in the region of the layer in question during 1972–74, but some thin layers could always be detected in the balloon photographs because of the great path length.

Zenith attenuation of solar radiation by the Fuego dust was hardly $>1\%$. Therefore the change of Earth temperature was $\sim 0.2 \text{ K}$ only⁸, and the effect on vertical transport less than that suggested by Scorer.

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- ¹ Scorer, R. S., *Nature*, **258**, 134 (1975).
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SCORER REPLIES—A characteristic of the occasion on which I observed the haze was that there were cirrus clouds above the tropopause and the tropopause itself was not apparent to the eye. After a year I would have expected purple twilight to have been much decreased, as the note says it was in April, and the large vertical extent of the haze suggested that it was not very old, otherwise it would have been sheared over into thin layers, of which several would be unlikely to be found together over one geographical location.

On the assumption that such layers come from volcanic eruptions, anyone determined enough can, of course, ascribe them to the one they think to be the most likely cause; but it is important to recognise that the assumption can dominate thinking about these phenomena. That explanation is not "more likely" except after acceptance of the assumption.

I do not see what attenuation of solar radiation or change of Earth temperature have to do with the radiative equilibrium, which I supposed was dominated by a change in the absorption of the outgoing long waves.

Reiter¹ gives a spread of residence times for stratospheric air ranging from 80 to 1,000 d at 20 km, which seems reasonable. Therefore to ascribe finality to any explanation of the haze is possible only if it can be traced accurately back to its origin. What I am suggesting is that there are probably many manifestations of the mass-interchange mechanism not yet properly investigated, and that hurricanes could be involved to an important extent.

¹ Reiter, E. R., *Rev. Geophys. Space Phys.*, **13**, 459 (1975).

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

A double life

J. W. Herivel

THE period covered by this volume witnesses the continuation, and exacerbation, of the controversy between Newton and Flamsteed, and the initiation of that with Leibniz over the question of priority in the calculus. Throughout the period Newton continues in his dual role as Master of the Mint and President of the Royal Society. His activities in the former role are copiously documented, those in the latter much less so. Compared with the previous period, and excluding the letters and papers relating to the Mint, there is perhaps a certain narrowing of Newton's correspondence in this volume. Thus there are now no letters to or from Locke or Halley. But any such restriction is more than compensated by the presence of the whole of the magnificent correspondence with Cotes over the preparation of the second edition of the *Principia*. Although Newton had long ceased to be mathematically or scientifically creative, the correspondence with Cotes proves that his intellectual energies remained undimmed. Apart from certain of the Mint letters, he also continues to write in a style marked by that concision, clarity, and strong sense of word order, first displayed in his 1672 paper on colours.

Almost all the Mint letters and memoranda are here published for the first time. On the whole, they are not the most interesting of Newton's letters, but there are exceptions, such as the memorandum (816a) "Of the assaying of Gold and Silver, and the making of indented Triall-pieces and trying the moneys in the Pix"; a measure of the importance which Newton himself attached to this memorandum is given by the fact that it exists in no fewer than eight drafts among the Mint papers. Among the letters relating to the Flamsteed controversy, a blistering letter (825) to Flamsteed about which



Machinery of the Mint, as illustrated in the Encyclopédie (Paris, 1771). The great screw-press (a fly-press in modern terms) which impressed the round blank.

Newton probably had second thoughts, and a hitherto unpublished letter (853) to Flamsteed from his "quondam friend and not yet profligate Enemy" Halley are noteworthy. Also another hitherto unpublished letter (978) to Flamsteed from a Committee of the Royal Society requiring the unfortunate Astronomer Royal to send them a copy of his observations for 1712 in accordance with "Her Majesty's Most Gracious Letter of 12 December 1710"—the warrant (reproduced at 814) extracted by Newton from the Government, requiring Flamsteed to surrender up his observations for any given year within six months of its ending.

Surprisingly few letters and memoranda relating to the Newton-Leibniz controversy are found in this volume. But as the editors point out in their introduction, this is no index of the time which Newton actually devoted to the controversy, inevitably at the expense of other activities including his correspondence with Cotes over the second edition of the *Principia*. When drawing up his celebrated edition of this correspondence, Edleston did not have access to the Portsmouth Collection. This accounts for the appearance of no less than 12 new letters between Newton and Cotes here published for

the first time. Taken together, these represent such a substantial addition to the Newton-Cotes correspondence as almost to call for a new edition, preferably with a fairly detailed exposition of Newton's lunar theory in an appendix. For although the introduction and notes to the present volume are very helpful in this respect, they still leave much obscurity occasioned in part by Newton's habit of carrying out long lines of reasoning without the use of symbols.

Among the new letters, number 829 throws interesting light on Newton's original intention to thank Cotes publicly for his vast (unpaid) assistance with the preparation of the second edition of the *Principia*. As suggested by D. T. Whiteside, the apparent cooling in Newton's relations with Cotes towards the end of the enterprise may possibly have been due to Cotes's failure to notice the error in Proposition 10 of Book II first drawn to Newton's attention by Johann [I] Bernouilli, (see 951a) in circumstances which the controversy with Leibniz rendered embarrassing to Newton. Of letters and memoranda on other miscellaneous topics the following, all hitherto unpublished, are noteworthy; number 802, in Newton's most pithy

The Correspondence of Isaac Newton. Vol. 5: 1709–1713. Edited by Rupert A. Hall and Laura Tilling. Pp. li+439. (Cambridge University: London, October 1975. Published for the Royal Society.) £20.

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style, with its triumphant announcement of his purchase of new premises for the Royal Society; number 878, in which Newton announces the death of Colonel Robert Barton to his widow's father leaving the latter "discretion to let her know it by such degrees and in such manner as may least afflict her"; number 918, of interest for Newton's views on gravitational attraction; and number 961a, an important memorandum *De Vi Electrica* concerned with Newton's views on attractive forces. The volume is brought to a fitting close by Bentley's letter (1002) to Newton announcing the publication of the second edition of the *Principia*.

No review of this volume would be complete without a tribute to the late J. F. Scott, editor of volume 4, whose untimely death, following so closely on that of H. W. Turnbull, was such a loss to this great project. It is to be hoped that the present editors will be able to carry it through to completion. Their editing of the present volume, including the footnotes and an admirable introduction, maintains the high standards set by preceding volumes, and makes it clear that no better choice of editors could be made for the project's final stages. □

Universal pictures

The Amazing Universe. By Herbert Friedman. Pp. 200. (National Geographic Society: Washington, DC, 1975.) \$4.25.

THE May 1974 issue of *National Geographic* included a feature on "The Incredible Universe"; this book develops the theme further with a new text by Herbert Friedman and a lavish use of colour illustrations. I am slightly disappointed, however, that the most has not been made of the opportunity to provide an account of the most intriguing theories of the new astronomy, and that the book largely rests on its illustrative laurels. Nevertheless, it is remarkably good value and helps to plug the gap between conventional 'popular' astronomy books and the revolutionary developments that have taken place in the past ten years or so.

Friedman has concentrated on discussion of the techniques of the new astronomy, which have opened up new parts of the spectrum and other areas to observation, but he does not complete the guided tour of theoretical work we have been led to expect. On solar neutrinos, for example, after

describing the Davis experiment, we are merely told that "if the cleaning-fluid trap produces no neutrinos, the mystery . . . may force solar physicists to re-examine their theories"; the "mystery" of Sco X-1 is described as "a continuing challenge for today's Eddingtons", when in fact the source now seems to be well understood; Weber's gravitational radiation claims are to be "interpreted with extreme caution", when there is now a clear consensus which the author does not report; and the mention of apparent faster-than-light separations in some QSOs is followed by the vague comment that "some ingenious explanations for this have been put forward", when those explanations seem both straightforward and a clear indication of the illusory nature of the measured super-light speeds.

Even the professional astronomer may enjoy the pretty pictorial record, and as far as it goes *The Amazing Universe* is good value both in appearance and scientific content. It would make an ideal gift for a young reader freshly interested in astronomy, with the proviso that the donor should take care to follow this up with more meaty texts if the bait is taken.

John Gribbin

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Photochemistry

Concepts of Inorganic Photochemistry. Edited by Arthur W. Adamson and Paul D. Fleischauer. Pp. ix + 439. (Wiley Interscience: New York and London, August 1975.) £13.30.

THIS volume gives an account of the developments in coordination photochemistry, each chapter being contributed by an authority in the field. Two chapters deal with photochemical processes: the first is a brief survey of the electronic spectroscopy of complexes, the second an account of the kinetics of photophysical processes based on concepts well established in organic photochemistry. Against this background are set chapters on charge-transfer photochemistry, substitutional photochemistry of first-row transition elements, and on the photochemistry of the heavier elements, of carbonyl complexes, of 1,3-diketone chelates and of simple inorganic ions in solution. In each of these chapters the existing experimental information on selected systems is critically reviewed and an attempt is made to interpret the data in terms of principles governing the reactivity of the excited state. The last two chapters are more speculative. The

first deals with photochemistry in the solid state, a challenging field of great importance in which the difficulties of obtaining quantitative information and of interpreting it, are formidable. The brief final chapter on photochromism and chemiluminescence concentrates on general principles. It includes the novel suggestion that the "thermally equilibrated excited state" in photochemical reactions may be identified with a particular modification of the "transition state" postulated in thermal reactions.

These are stimulating reviews which give an excellent account of the present state of a rapidly developing subject. Overlap between chapters has been kept to a minimum; a degree of uniformity in terminology and treatment helps to emphasise the common factors in different areas of the subject. A minor source of irritation, however, is the inconsistency in the use of units—the variety of captions for wavelength and wave number, for example.

The book will be welcomed by investigators in the wide field of inorganic chemistry. With its emphasis on concepts and interpretation it should be of general interest to many inorganic chemists.

M. I. Christie

Coherent calculus of subjective probability

Theory of Probability: A Critical Introductory Treatment, Vol. 2. By Bruno de Finetti. Translated by Antonio Machi and Adrian Smith. Pp. xviii+375. (Wiley: London and New York, 1975.) £10.50.

THIS is the second half of the English translation of Professor de Finetti's Italian *Theory of Probability*, and completes a work which, in the opinion of the foreword, "is destined ultimately to be recognised as one of the great books of the world". In reviewing the first half, besides sketching the background to the controversies which occupy the minds of many statisticians, I noted that it was the second volume which would carry Professor de Finetti's critical discussion of inductive reasoning and statistical inference, and that I awaited it with impatience (*Nature*, 251, 262, 1974). Now that it has arrived, it is slightly disappointing, although in other respects the author presents an invigorating and wholly delightful account of some fascinating topics in probability theory. In treating these (chapters 7-10) he greatly strengthens his Bayesian position by his deft treatment of paradoxical examples

(it would be too much to call these teasers of probability theory *paradoxes*). Studded with illuminating asides and revealing anecdotes, this part of the book is a delight. How many statisticians know that the points of inflexion of the normal distribution are at the one-standard-deviation points? Professor de Finetti knows that the tangents at these points cut the axis at the two-standard-deviation points, and such asides give the book its flavour.

Chapter 11, 'Inductive Reasoning; Statistical Inference', is the kernel of the book. "It is particularly instructive to consider the process by which new scientific theories are formulated. The first step is an intuitive one, arising out of some particular set of observations, but then various modifications are made as a result of more up-to-date results which suggest that this or that alternative theory provides a better explanation". So far so good, but what is to be the currency of explanation? I would say *likelihood*, but for de Finetti: "In essence, it is always a question of analysing the current state of information by means of Bayes's theorem". Later, "In our formulation, the problem of induction is, in fact, no longer a problem: we have, in effect, solved it without mentioning it explicitly. Everything reduces to the notion of conditional probability".

The author presumes on our acquiescence, and we look in vain for arguments which will lead the doubting majority to join the congregation of the Reverend Thomas Bayes: why should scientific hypotheses be treated like statistical events? If they should be, then the Bayesian position surely follows, for I believe de Finetti carries that part of his case—that the right calculus for events of uncertain outcome is the coherent calculus of subjective probability: it is, perhaps, his major contribution. Bayes, I think, would have approved; indeed, de Finetti could with profit have included an analysis of Bayes' paper in his book, for the one presages much that is in the other.

In fact, Bayes did not include scientific hypotheses in his scheme: that proposal is neo-Bayesian. But whatever we call it, it is the crucial issue. For me it is neither a reflection of how science does progress nor a paradigm for how it should. If universally applied it would reduce creative scientific activity to the level of accountancy. Coherence has its place, just like the double-entry book-keeping introduced by de Finetti's compatriots—the Lombard bankers of long ago: *c'est magnifique, mais ce n'est pas la guerre*.

But let each reader decide for himself. de Finetti's two volumes are essential reading. **A. W. F. Edwards**



Bowl (diameter 30 cm) found as part of a grave lot. New Mogollon pottery style of the 10th century AD, Mimbres area of south-western New Mexico. Decorated with a black-on-white design of enigmatic human figures, possibly representing the contrast between life and death or male and female. The hole puncturing the base, 'killing' the object, helped release the vessel's spirit into the next world. Taken from *The American Indians: Their Archaeology and Prehistory*. By Dean Snow. Photographs by Werner Forman. Pp. 265. (Thames and Hudson: London, April, 1976.) £6.50.

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Edward Arnold

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The Blood of Sheep: Composition and Function. Edited by M. H. Blunt. Pp. xiii+224. (Springer: Berlin and New York, 1975.) DM 72; \$31.

THIS is not a haematological text: the leukocytes receive scant attention, and the platelets and coagulation do worse. Instead it is a collection of chapters by different authors on body fluids, cells, carbohydrate and lipid metabolism, minerals, hormones, genetic markers, haemoglobins, immunoglobulins and anaemias. Each chapter is a factual review with little discussion, and occasional overlap with its fellows. More discussion would have been welcome and more selective editing could perhaps have provided the space. There seems little need in a work of this kind for a description of the actions of each hormone, irrespective of what is known about its presence in the sheep. The chapters vary between crisp, direct succinctness and a lengthy struggle to be comprehensive, and each provides numerous useful references. Nevertheless, because of its layout and index the book proves difficult to use as a reference work. Such criticism should not detract from its usefulness in bringing together a great deal of very interesting material for those centres where the sheep is a major object of study, and their libraries should acquire it.

The individual worker who confines himself to a single aspect of the sheep may find it of only ephemeral interest, and it is too expensive for that. It will prove even more expensive if he injects either of the two doses of adrenaline quoted incorrectly on page 33 from two quite separate sources. Each is a 1,000 times too large.

Brian Greenwood

Optics and Its Uses. (Modern University Physics Series.) By G. F. Lothian. Pp. 218. (Van Nostrand Reinhold: New York and London, September 1975.) Cloth £7.50; Paper £3.85.

DERIVED from a lecture course, this book is an undergraduate text which includes mention of modern developments such as lasers, non-linear optics, holography, integrated optics, Fourier transform spectroscopy, fibre optics, spatial filters, and so on, as well as dealing with basic theory. In a text of this length it is impossible to cover this very wide range of topics in depth, and reference to some is almost in note form. Although the consequent large number of section and sub-section headings break the flow in places, the arrangement should be helpful to a student, enabling him to make easy reference to a

particular topic. In proceeding to an honours degree it would be necessary, as the preface suggests, for a student to supplement his reading, and most chapters refer to specialised books. The reader is expected to work through examples which are contained in relevant places throughout the book. As the title indicates there is emphasis on optical instruments and equipment; and understanding these is helped by a large number of good drawings, photographs, and tables. The latter illustrate a particularly useful feature of the book in providing an unusual amount of numerical data which would not otherwise be easily available to a student.

W. J. Bates

Books brief

Lord Rutherford on the Golf Course. By F. G. Mann, Trinity College, Cambridge, UK. Pp. 33+6 plates. (Published privately.) £1.50, UK; \$4.00, Overseas and US. Copies from the author.

AN accumulation of anecdotes, most, but not all, about Rutherford and golf. An absolutely bizarre story about Aston and a rice pudding. Nicely produced, but present-day prices hardly help such ventures even when published privately. □

Applied Geophysics. By W. M. Telford, L. P. Geldart, R. E. Sheriff and D. A. Keys. Pp. xvii+860. (Cambridge University: Cambridge and London, January 1976) £26.

THE book is a very good one, written and illustrated with clarity. It sets out what is done in the present day in the application of geophysics to exploration. For each method the theory is given in sufficient detail for understanding that method, with the mathematical development to support it. Implementation of the method is described, with practical examples. Further problems are given for the reader to solve, and each method has a good bibliography.

Two large sections of the book have special merit. A long chapter on the seismic method is a unified account of the sophisticated technology to which many have contributed time and money: it is almost a single line of development. By contrast the chapters on electrical, electromagnetic and related methods bring together a great diversity of methods

and practice under a common theoretical and descriptive treatment. Chapters on other methods are written with the same authority and clarity; the exception is, perhaps, the chapter on well-logging. This gives the impression of being an afterthought; and it suffers by comparison with the rest.

There are a few criticisms. The writers do not distinguish everywhere between interpretation and modelling, the inverse process. Time domain sampling at 4 ms intervals does not correspond with frequency domain sampling at 250-Hz intervals (p375). The noun 'practice' is not spelt with an s.

The book should fill the requirements of university teaching and of company training programmes. It is of value to professional geophysicists who can rarely keep up with geophysical literature outside their speciality.

A. A. Fitch

An Introduction to Non-Numerical Computing. By Patrick A. V. Hall. Pp. 193. (MacDonald and Janes/American Elsevier Computer Monographs.) £2.95.

THIS is a very competent collection of methods, algorithms and exercises divided into chapters on "Abstract Information and Algorithm Structures", "Machine Structures", "String Processing", "Files and Tables" and "Sorting". The book forms a reliable textbook of available techniques; it does not break any new ground but current thinking is well expounded. Hall's views on flow-charting are well worth noting. My only criticism is that a book on non-numerical computing should adopt a style different from mathematical texts. It is not that the mathematics is difficult—it has, in fact, been kept simple—but the flavour is still mathematical. References are also made to details of earlier chapters although the author says that the later chapters can be read in any order. The way in which the algorithms are presented also causes some difficulty, being a mixture of precise ALGOL-like instructions and English statements: but one soon adapts. The book is intended to support a second course in computing and leans heavily on ALGOL 60. Perhaps it could more usefully be based on ALGOL 68; it would appeal to a wider audience if it had been based on COBOL. The work is well illustrated and indexed. The exercises at the end of each chapter, although perhaps a little difficult for second course students, will be extremely valuable for teachers.

R. D. Parslow

obituary

Werner Heisenberg died on February 1, 1976. His youth was inspired; he lived through a long war which destroyed cities, lives and the spirit of people; in his old age he tried to rebuild a great tradition which had been virtually wiped out by Hitler.

The beginning of this century was the most remarkable period in the history of science. It was, in fact, so revolutionary that most people, including the majority of intellectuals, have failed to grasp what has happened. The leaders of this revolution were Heisenberg and his great teacher, Niels Bohr. The result was a complete explanation of the world of the atom. This fact is known. But the philosophical implications are all but forgotten. The philosophical principle is called 'Complementarity'.

Complementarity, or its older designation, dualism, is not new. It is as old as man. What are you: a piece of matter or a soul? It is difficult to deny either body or spirit. But to believe in both was considered sheer mysticism by a generation of scientists who lived in a world in which truth seemed to be simple.

It was Bohr who introduced a peculiar element into the most straightforward of all experimental sciences, physics. He based his quantum theory not on an explanation but on a contradiction. His ideas caused consternation, but his successes could not be denied.

Heisenberg, barely 24 years old, created order where there seemed to be chaos. Where Bohr navigated a new ocean keeping the shore of classical physics in sight by the use of the correspondence principle, Heisenberg left the old land and sailed beyond the point of no return. He found the New World of quantum mechanics. (It is even more remarkable that in inventing 'matrix mechanics' he did not know anything about matrices—he re-invented them.)

The unity of physics was re-established by the cooperation of Heisenberg and Bohr. They asked the essential question: are atoms made of waves or particles? The answer: we cannot explain atoms without using both the wave picture and the particle concept. The two can be reconciled by setting limits to the accuracy in applying either description. For instance, the more accurately we know the position of a particle the less accurately can we know its velocity (or momentum). This is Heisenberg's 'Uncertainty Relation'.

His first illustration was based on the resolving power of the microscope, a point on which, amusingly, he was ignorant at his PhD examination.

What has this to do with the body-spirit dualism? Heisenberg has shown that two obviously contradictory concepts, waves and particles, are not only necessary in explaining the structure of atoms but also, these two concepts can co-exist within a single rigorous mathematical formalism. If this can be done today for atoms why should it not become possible to do something

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similar someday in describing life or even a human being? Whether anything of the kind will happen one cannot tell. But Bohr and Heisenberg did something which even in the context of the great intellectual accomplishments of modern science is quite exceptional.

One often has the feeling that discoveries are just waiting to be made. Most scientists, even the great ones, merely accelerate science. They don't change it. Bohr and Heisenberg gave a completely new turn to the understanding of the world. Without them this understanding may never have come, at least not in our present culture. (This may sound like an exaggeration but I remember Bohr's statement: "If I can't exaggerate, I can't talk.") Without them the science of the atoms may have developed into a formalism even more remote from the common, un-mathematical understanding than it is today. Without them the intricate concept of the interaction between the observer and the object may have been missed. (Heisenberg modestly denied this; he said that he only found what was there.)

Of course, the prime mover was Bohr. He was far ahead of everybody, but he was obscure, almost oracular. It was Heisenberg who brought the wisdom of Bohr (which Bohr managed to hide in his own words) to the level of lucidity where the common-or-garden theoretical physicist and mathematician could understand it, if he tried.

But in performing this translation of philosophy to exact science, Heisenberg did not lose any of the great original concepts. The philosophy (not coming from a philosopher) might have been lost. The science will survive. This is why Heisenberg is one of the few who have truly changed the world. It can never change back.

Heisenberg was made a professor when he was 26, and I became one of his students. We all talked physics, and played ping-pong and chess, and in this close environment a lot of fundamental work was done. Heisenberg received the Nobel prize in 1932 for the historic discovery of quantum mechanics and its implications, but much of his additional work would have sufficed in its own right to earn that distinguished honour. Some milestones are: papers on quantum field theory (QED with Pauli (1929, 1930) and divergence problems (1934)—Heisenberg's positivism can be exaggerated!), work on the solid state (the first understanding of ferromagnetism (1928)) and on symmetries (the distinction between ortho- and para-helium and hydrogen) and the invention of iospin (1932). We knew that the world was open and the key was reason.

Then came Hitler and reason was no more. In the summer of 1939, Heisenberg visited the US. Several of us asked him to stay. His answer to me was: "If your brother had stolen a silver spoon, he is still your brother." It was much more than a silver spoon. Both of us knew it, but neither of us could say so.

During the war Heisenberg worked on atomic energy. He used his position to save the lives of several fellow physicists. In his project he got nowhere. Why was Heisenberg unsuccessful in his wartime job? I am sure that the reason was not any lack of ability. I believed firmly that the obstacle was the silver spoon.

In his last years he wrote the story of his great experiences in science and the story of the tragedy of the deeply troubled times in Germany. In his writ-

ings, it becomes clear why Heisenberg failed during the war. He was happy to see that the technical difficulties were enormous and he concentrated on peaceful reactors. He only hoped that the American scientists, including the refugees from Europe, would come to the same conclusion. In 1941 he visited Bohr to give him this message. But Bohr's ears were closed and Heisenberg knew that he must continue to live in a small island surrounded by the Nazi horror; the greater world was too far away; even his best friend had turned his back. Bohr later escaped to the US, but Heisenberg's message was never delivered.

I shall not forget one of Heisenberg's recollections. With a colleague, he is hurrying through burning Berlin, trying to get back to two of his sons when his shoe catches fire. And while he puts out the fire, he discusses with his friend how science will be rebuilt in Germany when the catastrophe has run its course.

Shortly after the defeat of Germany, he hears of Hiroshima. He can hardly believe it. He is convinced that a political movement, or country, must be judged by its methods, not by its professed aims. (Heisenberg never men-

tions a nightmare which must have occurred to him: what would have happened if atomic bombs had been used against Germany. Hitler, unlike Hirohito, would not have had the fortitude to surrender. Hitler wanted Germany destroyed if the war was lost.)

But at last peace came and the reconstruction started. Heisenberg returned to the land he loved most, Bavaria. He continued to work on the hardest and most exciting problems of physics. The same basic philosophy that had led him to his formulation of quantum mechanics took him to the S-matrix approach to particle physics (1946). He later devoted himself to a bold attempt to understand particle physics through non-linear field theories with an inherent 'length' scale—continuing, in fact, pre-war ideas. This expressed his opposition to the idea that there are never-ending hierarchies of successively more fundamental particles—the 'big fleas—lesser fleas' syndrome. He also initiated the German programme for the production of nuclear energy.

In a long, magnificent and difficult life he never lost his sense of purpose, nor a sense of humour. On the 800th anniversary of the Bavarian state, he appeared on television and said: "The

Bavarian unites the discipline of the Austrian with the charm of the Prussian." Most particularly, he retained a sense of balance. To each serious argument, he attached its opposite. He understood, from his own physics that between 'yes' and 'no' there are possible answers, less abrupt and more fruitful.

I am one of the relatively few who had a teacher like Heisenberg. From his life there remain for me two lessons. One is that the cataclysm of yet another world war must be avoided. Next time, though man will surely survive, it will be even more difficult to imagine how the spirit can be resurrected. The other is that the path of peace is not only difficult but also uncertain. No simple proposal, neither power nor appeasement, will suffice.

The title of Heisenberg's memoirs *Der Teil und das Ganze* means literally the part and the whole: the world cannot be divided into science and politics, nor into any other components. It is strange and wonderful that this lesson of unity should have been taught by a physicist who worked on a subject that most consider remote from the common understanding.

Edward Teller

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